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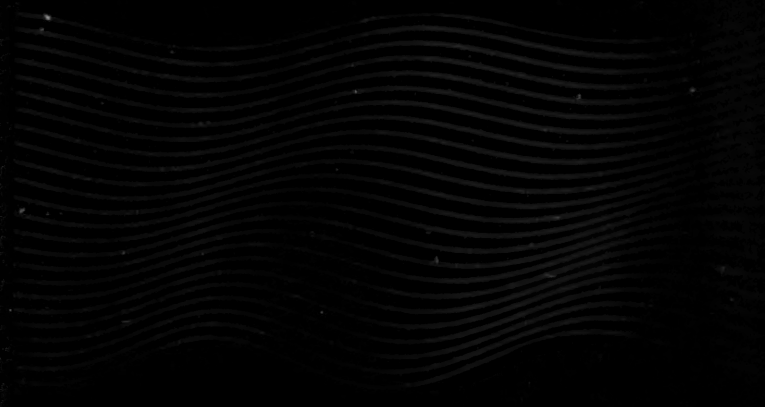

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Nonlinear Feedback Dynamics in Fisheries: Analysis of the Deriso–Schnute Model

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Fishery models of the Deriso–Schnute form are based on the dynamics of an age-structured population, together with a nonlinear stock–recruitment relationship. Cyclical dynamics are commonly observed in fisheries and have been attributed to feedback between stock and recruitment. In this paper, we do four things. First, we present analytical results on sustained oscillations driven by nonlinear recruitment. These results show explicitly how density dependence near equilibrium determines the character of sustained population oscillations. Second, we briefly characterize the dynamics of the Deriso–Schnute model when the density-dependent response becomes very strong. We find that the Deriso–Schnute model displays sustained, complex (probably chaotic) variability of large magnitude, but only typically when reproduction is concentrated at very few ages. Third, we dissect the nature of density dependence in recruitment, contrasting a "local" view that uses information about response to small variations in stock with a "global" view that uses a function such as Schnute's over the entire range of stock levels. Finally, we argue that the global approach leads to practical and theoretical difficulties and that a local view may be more biologically realistic.

Les modèles de pêche de type Deriso–Schnute sont fondés sur la dynamique d'une population à structure par âge, et avec un rapport stock–recrutement non linéaire. Les pêches ont souvent une dynamique cyclique et celle-ci est attribuée à une rétroaction entre le stock et le recrutement. Dans le présent article, nous avons établi quatre points. Premièrement, nos résultats analytiques portent sur des oscillations soutenues dues à un recrutement non linéaire. Ces derniers montrent explicitement comment la dépendance à l'égard de la densité près du point d'équilibre détermine le caractère des variations démographiques soutenues. Deuxièmement, la dynamique du modèle Deriso–Schnute a été brièvement caractérisée lorsque la réaction de dépendance à l'égard de la densité est devenue très forte. Nous avons constaté que ce modèle présente une variabilité complexe et soutenue (probablement chaotique) de grande amplitude, mais en général seulement lorsque la reproduction est concentrée sur très peu de groupes d'âge. Troisièmement, nous avons décortiqué la nature de la dépendance à l'égard de la densité du recrutement, en opposant une vue « locale » qui utilise des données sur la réaction à de faibles variations du stock, à une vue « globale » qui utilise une fonction comme celle de Schnute pour toute la gamme des niveaux de stock. Finalement, nous soutenons que l'approche globale entraîne des difficultés pratiques et théoriques et qu'une vue locale peut être biologiquement plus réaliste.

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1. Introduction

Many commercially important fish stocks are highly variable over time, a feature nicely illustrated by the time-series of recruitment for North Atlantic species compiled by Myers et al. (1990). If we examine two of the longer series they report, for cod and haddock, recruitment appears to oscillate irregularly over time, an impression supported by their estimated autocorrelation of recruitment. Other examples are found in their report; temporal variation is also of great interest in other marine species such as crabs (Botsford 1986) and barnacles (Roughgarden et al. 1985).

Such variability has obvious practical significance and raises at least three scientific questions. First, is variability evidence for or against natural regulation of fish abundance (Shepherd and Cushing 1990)? Second, do populations vary largely in response to environmental variability and, if so, to which environmental variables (Koslow et al. 1987)? Third, is population variability driven by density-dependent interactions between individuals (Levin and Goodyear 1980; Levin 1982)? These questions cannot be cleanly resolved by empirical information: available data cover relatively short time spans, and most populations of interest are spatially dispersed and incompletely monitored. These questions are not unique to marine species. A vigorous and essentially similar debate goes on in terrestrial ecology (Strong 1986, 1988; Hassell et al. 1989). Progress in answering these questions will require careful analysis of the properties and assumptions of the models and methods that underlie analyses of data.

This paper contributes new results about the dynamic properties of populations with time-delayed recruitment, results that generate useful insights into the nature of density-dependent regulation. We analyze the nonlinear age-structured fishery model of Deriso (1980) as extended by Schnute (1985). This model, which provides a general, systematic framework for modeling fish populations, has been shown to be useful in several ways (Kimura 1985, 1988; Schnute 1985). This model is also closely related to models for the Dungeness crab (Botsford 1986), striped bass (Levin and Goodyear 1980), and even human fertility change (Lee 1974; Wachter 1991b). Our results apply to all models that are a special case of the Deriso–Schnute family.

The oscillatory propensity of such models was discovered by Ricker (1954). Recent analyses (studies cited above, and especially Bergh and Getz 1988) have expanded on Ricker's work by studying population numbers very close to equilibrium, where the model is effectively linear. Linear stability depends on the strength of density-dependent regulation at equilibrium; strong enough regulation makes the linearized version of the nonlinear model unstable with oscillatory solutions, suggesting that the full nonlinear model might also display oscillations. However, sustained oscillation requires a combination of linear instability to kick populations away from equilibrium and nonlinear regulation to keep them in check along some cyclical trajectory. Therefore, the linear analysis provides no information on the amplitude and pattern of oscillation, although it has been suggested (Levin and Goodyear 1980; Botsford 1986) that the linear results might approximate the period of oscillation.

In this paper, we do four things. First, we present analytical results on sustained oscillations driven by nonlinear recruitment. These results show explicitly how density-dependent nonlinearities in the Deriso–Schnute model determine the character of sustained population oscillations. The quantitative and qualitative insights that flow from this nonlinear analysis are important in examining hypotheses about observed population variability. The methods we develop are straightforwardly applicable to models of nonlinear population recruitment, and we urge their wider use. The limitation of our nonlinear analysis is that it is "local," that is, we are concerned with density dependence as population varies by about 10–50% of its equilibrium level. In nature, populations may fluctuate over a much wider range, in which case the "global" properties of density dependence become important. Large fluctuations may be caused by external forces acting on the population (an El Niño, say) or by strong density dependence that causes large swings in population size.

Second, we briefly characterize the nonlinear dynamics of the Deriso–Schnute model when the density-dependent nonlinear response becomes very strong. We find (as did Botsford (1992) in a related but much simpler model) that the Deriso–Schnute model displays sustained, complex (probably chaotic) variability of large magnitude, but only typically when reproduction is concentrated at very few ages.

Third, we dissect the nature of density dependence in recruitment. There are two, essentially polar, ways of looking at recruitment. Traditionally, a "global" recruitment function is hypothesized to apply at all population sizes. Such a function couples the strength of density regulation near equilibrium to density response at much lower and higher population sizes. We argue that the global approach leads to practical and theoretical difficulties and should be used with reservation. An alternative picture of recruitment is that it is "local" in character, as in Strong's (1984, 1986, 1988) advocacy of "density-vague" regulation. A local approach may be more consistent with the data, while providing a nonlinear mechanism for population oscillations, as in our analysis here. The local versus global issue appears in a different guise in the debate about the existence of regulation in fisheries (e.g., Shepherd and Cushing 1990).

Schnute (1985) presented an especially clear derivation of the model we use, so we provide no derivation here. Section 2 presents the model and analyzes its dynamic features. The analysis will be somewhat unfamiliar to many readers, but we concentrate on providing a clear passage through the main steps. Following that section, we present results that concern very strong density effects. Next we turn to the issue of global and local density regulation. Finally, we consider the inverse question of inferring mechanisms from data and the utility of our results.

The dynamic analysis presented here draws on mathematical results in the bifurcation theory of Cushing (1978) that were extended by Tuljapurkar (1987) to provide analytical formulas for characteristics of the nonlinear dynamics. These formulas were applied by Wachter (1991b) to study human population cycles. We have extended these formulas by using the results of Staffans (1987).

2. Dynamics of the Deriso–Schnute Model

This section sets out the model, presents equations for biomass dynamics, defines and describes the linear stability boundary, and then examines sustained oscillations.

2.1. The Model

The model describes a population at discrete time intervals, usually a year. Individual fish are assumed to become mature at age τ and at the same age enter the fishery as recruits (this is "knife-edge" recruitment). At ages greater than 1, the survival rate per time unit is a constant s. The weight of fish changes according to the Brody growth law with a growth constant ρ; letting W_a be the weight at age a:

$$\frac{W_a}{W_\tau} = V + (1-V)\left(\frac{1-\rho^{a-\tau+1}}{1-\rho}\right).$$

The parameter V plays the role of an intercept in this relationship; in Deriso (1980), V was set to zero whereas it was nonzero in Schnute's (1985) more general discussion. The dynamic variable of the model is the total stock biomass, written as B_t at time t, and equal to the total mass of mature fish. The final ingredient in the model is the recruitment, the mass of fish reaching age τ, which is written as R_t at time t. The renewal equation for population numbers, when transformed to biomass, is

$$(1) \qquad B_t = \sum_{a\geq 0} s^a \frac{W_{\tau+a}}{W_\tau} R_{t-a}.$$

Schnute (1985) showed that this renewal equation can be summed into more compact form. The model is completed by positing a recruitment function that relates the biomass R_t of recruits to the stock biomass B_t:

$$R_t = F(B_{t-\tau}).$$

Schnute used the recruitment function

$$(2) \qquad R_t = \alpha B_{t-\tau}\,(1-\gamma\beta B_{t-\tau})^{1/\gamma}.$$

The constant β here must necessarily have dimensions such as per kilogram. For convenience, we measure biomass in units of $1/\beta$, so that we have one fewer parameter to deal with. The parameter γ in equation (1) is a shape parameter; as it varies, equation (2) can be made identical to such standard recruitment functions as the Ricker and Beverton–Holt. Equation (2) is an example of a "global" stock–recruitment relationship, and it implies a relationship between density dependence at low and high stocks. We return to this matter later.

Substantial parts of our discussion apply to an arbitrary recruitment function F. When we deal specifically with the function in equation (2), we will say so.

2.2. Equilibria and Dynamics

A constant equilibrium of equation (1) is a biomass B^* at which recruitment just balances mortality loss. The dynamics away from equilibrium are described by setting $x_t = B_t - B^*$ and writing the density dependence in recruitment, by Taylor expansion, as

$$(3) \qquad R = F(B^* + x) = F(B^*) + F_1 x + F_2 x^2 + F_3 x^3 + \text{etc.}$$

where "etc." stands for higher-order terms. The successive coefficients in equation (3) are successive derivatives of the stock–recruitment function evaluated at B^*; thus, at $B = B^*$:

$$F_j = \frac{1}{j!}\frac{d^j F(B)}{d B^j}.$$

Equation (1) in turn can be written as

$$(4) \qquad x_t = L_t(x) + Q_t(x) + T_t(x) + \text{etc.}$$

where L_t, Q_t, and T_t are, respectively, linear, quadratic, and third-order in x. For example:

$$L_t(x) = F_1 \sum_{a\geq 0} s^a \frac{W_{\tau+a}}{W_\tau} x_{t-a}$$

$$Q_t(x) = F_2 \sum_{a\geq 0} s^a \frac{W_{\tau+a}}{W_\tau} x^2_{t-a}.$$

Equation (4), including all terms labeled as "etc.", is just the original equation (1). The value of (4) is that it reveals the dynamic consequences of density dependence for biomass values in successively larger neighborhoods of B^*.

2.3. Threshold: Boundary of Linear Stability

Previous analyses (Levin and Goodyear 1980; Bergh and Getz 1988) suppose that x_t is very small, so that one can ignore all but the first term in (4). Density dependence is reflected in the resulting linear equation only because L_t contains as a factor the slope F_1. As a linear difference equation,

$$(5) \qquad x_t = L_t(x)$$

has exponentially changing solutions. The solution that grows most rapidly has the form $C\lambda^t$, for some constant C. If $|\lambda| < 1$, an initially small x_0 will over time shrink to zero: the equilibrium biomass B^* is linearly stable. Previous studies of many special models have shown that, as the slope-at-equilibrium F_1 is made increasingly negative, we find $|\lambda| > 1$, so that an initially small x_0 will grow over time. But what happens to the biomass as it becomes increasingly different from B^*? The answer lies in the nonlinear terms in (4) because as x_t increases, the quadratic term Q_t, the term T_t, and so forth, will become impossible to ignore.

Before discussing the nonlinear dynamics of x_t, consider the following question: for the linear part of the Deriso–Schnute model, is it always true that linear instability will occur for some negative value of the slope F_1? Wachter (1991a) proved that the answer is yes (his results apply to many other models as well). This means only that if we insert a sufficiently negative value of F_1 in the linear equation (5), we will get an exponential solution that grows. It need not be true that all stock–recruitment functions are capable of having sufficiently negative slopes at equilibrium; indeed, this is not always true for the Schnute function, as shown below.

How strong does density dependence have to be to produce linear instability? We use the term threshold to identify parameters that put the linear model on the edge of stability, i.e., at which $|\lambda| = 1$. The value of F_1 at threshold depends on the demography of the fish: here, the demographic parameters are contained in the age pattern of the coefficients

$$(6) \qquad h_a = s^a \frac{W_{\tau+a}}{W_\tau}.$$

These h_a are an "influence function" in the sense of Botsford (1986), and they influence the location of the threshold for equation (5). The age pattern of the h_a can be summarized by the moments of the distribution $\{h_a/H\}$, where $H = \Sigma_a h_a$. The mean, μ, is approximately equal to τ, the age at recruitment; μ is the length of a "generation" in our population. The variance, σ^2, and the third moment, μ_3, depend on the relative size of the growth parameter ρ and the annual survival probability s. At threshold, we may set

$$(7) \qquad \lambda = \exp(2\pi i / T_0), \text{ where } i = \sqrt{(-1)}.$$

The threshold (see Appendix A) is approximately given by

$$(8) \qquad \hat{F}_1 = -\left(\frac{1}{H}\right)\exp\left(\frac{2\pi^2\sigma^2}{T_0{}^2}\right)$$

$$T_0 = 2\mu - \frac{\pi^2 \mu_3}{3\mu^2}.$$

When the slope of the recruitment–stock function at equilibrium is more negative than the $\hat{F}_1$ in (8), we should find linear instability. The relationship (8) is only approximate, but we have found it qualitatively useful over a wide parameter range.

In (8), T_0 is the period of oscillation of the linear model (5) exactly at threshold. In other words, when we are at threshold, the linear model will support oscillations of any amplitude and this period. These oscillations are not solutions to the nonlinear model. Their period, from (8) (and as noted by many earlier studies), is very nearly equal to twice the generation time.

2.4. Getting to Threshold: Schnute Function

The effect of biological parameters on the linear stability threshold for the Schnute recruitment function (2) may be

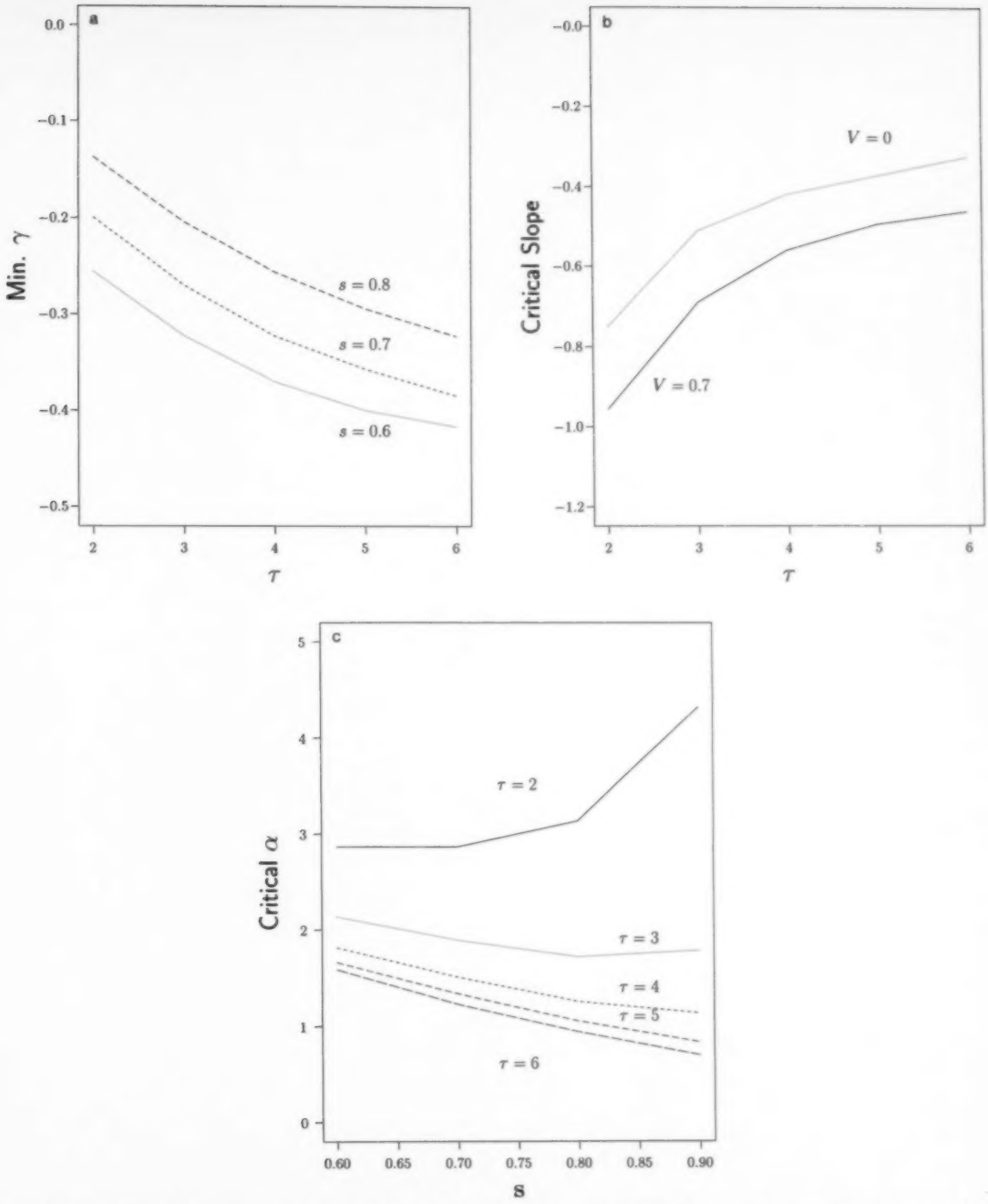

FIG. 1. (a) Minimum value of γ required in Schnute's function to make instability possible. Parameters are $V = 0.7$, $\rho = 0.7$, and $\alpha = 0.5$, and the curves in ascending order have $s = 0.6$, 0.7, and 0.8. (b) Effect of intercept parameter in Brody curve. Critical slope $\hat{F}_1$ for the Deriso model $V = 0$ in lower curve and Schnute model with $V = 0.7$. (c) Effects of time lag and adult survival on stability threshold. Here, threshold is reached by increasing α in the Schnute function. Solid curve is for $\tau = 2$; viewing the right-hand end of the curves, the value of τ increases through 2, 3, 4, 5, and 6 in descending order. Other parameters are as in Fig. 1a.

explored by inserting (7) into the linear equation (5) and solving for $\hat{F}_1$ and T_0. Bergh and Getz (1988) did just this for the Deriso model, in which the mass of fish just before recruitment is set to zero. Our interest here is in nonlinear dynamics, so we will only discuss some key features of the threshold. The parameter "space" of the model consists of the quantities τ , s, ρ, V, α, and γ.

The slope F_1 of the Schnute function at equilibrium B is

$$\left(\frac{1}{H}\right)\left[1-\left(\frac{(\alpha H)^{\gamma}-1}{\gamma}\right)\right].$$

When $\gamma > 0$, this can be made as negative as desired by increasing the product αH. When $\gamma < 0$, this slope decreases to an asymptote as αH increases. Thus for a particular choice of the demographic parameters h_a, there will be a minimum value of γ that is required in order to reach the stability threshold. This is important because the value of γ for a particular fishery may well be too low for threshold to be achievable. For exampmle, Deriso's (1980) estimate for three fisheries yields γ values around -1. For comparison, we show in Fig. 1a how the minimum value of γ changes with the survival rate s and the age at recruitment τ.

The threshold slope $\hat{F}_1$ depends also on the shape of the influence function h_a. The demographic parameters that determine this for the Deriso–Schnute model are restricted as follows: clearly, $0 < s < 1$ and $0 \leq V < 1$; it is biologically sensible to assume that $h_a \to 0$ as $a \to \infty$, which implies that $s\rho < 1$. We consider only values of the age of maturity $\tau \geq 2$. Increases in the survival parameter s, the growth curve intercept V, and the growth parameter ρ all increase the dispersion of h_a, so that its variance will increase. Biologically, increases in these parameters correspond to an increased averaging over past cohorts in (1). From (8), such increases also raise the magnitude $|\hat{F}_1|$ of the threshold slope, meaning that it takes greater density response to achieve linear instability.

Two other aspects of the threshold deserve note. First, the Deriso and Schnute versions of model (1) differ because the former sets the growth curve's intercept parameter $V = 0$. The difference in threshold when $V > 0$ is small for small V; as V increases towards 1, there is some change in $\hat{F}_1$ but the change is sizeable mainly when $\tau = 2$ (see Fig. 1b). This brings us to our second point, that decreases in survival rate lead to an increase in the threshold slope (see Fig. 1c).

We have examined the threshold numerically for a wide range of the model parameters and find that $\hat{F}_1$ is typically in the range -0.1 to -1; when $\tau \geq 5$, it is typically in the narrower range -0.4 to -0.6. These values imply an increase in recruitment (near equilibrium) of between 0.1 and 1 kg of fish for every 1-kg decrease in stock, in order to achieve the threshold of linear stability. For the Schnute function, we may compare these numbers with the recruitment levels when stocks are small. Taking stock to be very small in (2) shows that recruitment is α kilograms per kilogram of stock. Various estimates in the literature (Ricker 1954; Cushing 1971; DeAngelis and Christensen 1980; Deriso 1980; Sheperd 1982; Kimura 1985, 1988; Maccall et al. 1985; Gómez Muñoz 1986) for commercial fisheries suggest that α is in the range 0.1–3. Thus, if we think of density dependence simply in terms of the local change of kilograms of recruitment per kilogram of change in stock, the stability threshold requires density dependence only a few times larger than the recruitment rate per kilogram of stock at low density.

2.5. Beyond Threshold: Nonlinear Dynamics

To examine the dynamics of equation (1) when the linear equation (5) has unstable solutions, we use bifurcation theory. A simple, general account of this is found in Nicolis and Prigogine (1989), while a technical, general account is given in Guckenheimer and Holmes (1983).

Suppose that for a particular fishery and stock–recruitment function, we have identified the threshold. We now ask: what are the dynamics of equation (1) if that recruitment is made sufficiently sensitive to density so that $F_1 < \hat{F}_1$? To achieve this level of density dependence, we will suppose that F_1 is a parameter that can be changed. For example, in the Schnute function, F_1 increases when α increases. Setting $F_1 = \hat{F}_1 + f$ with $f < 0$, we know that the solution of the linear equation (5) will now be unstable. That is, there will be solutions of the form $C\lambda^t$ where $|\lambda| > 1$. Since λ is given by equation (7) when $f = 0$, for small f the unstable solutions will have

$$\lambda = \exp\left[\left(\frac{2\pi i}{T_0 + \delta_1}\right) + \delta_2\right]$$

where δ_1 is the change in the period of oscillation, and $\delta_2 > 0$ because the solution is unstable. From the standard theory of linear equations, we expect these δ's to change smoothly with f.

The nonlinear terms in (4) will determine the fate of such linearly unstable biomass change as soon as biomass starts to differ significantly from B^*. If the nonlinear terms stabilize the biomass, we expect that there will be a solution of the nonlinear equation (4) with a period $T(f)$. Nonlinearity implies that a simple exponential solution will not work; instead we may represent the solution as a Fourier series:

$$\begin{aligned}(9)\qquad x_t = {} & A\exp(2\pi i/T(f)) + A\exp(-2\pi i/T(f)) \\ & + B_2\exp(2\pi i/\{2T(f)\}) + B_0 \\ & + B_{-2}\exp(-2\pi i/\{2T(f)\}) \\ & + [\text{terms of period } 3T(f), 4T(f), \text{etc.}].\end{aligned}$$

The coefficient A will be called the amplitude of this solution and will depend on the level of density dependence, so that $A = A(f)$. Bifurcation theory allows us to compute the amplitude $A(f)$, the period $T(f)$, and the form of the oscillations via the coefficients of successive terms in the Fourier expansion (9).

When density dependence is just a little over threshold, so f is small, bifurcation theory yields coefficients a_1 and b_1 such that

$$(10)\qquad f = a_1 A^2$$

and

$$(11)\qquad \left(\frac{2\pi}{T(f)}\right) = \left(\frac{2\pi}{T_0}\right) + b_1 A^2.$$

These coefficients characterize sustained nonlinear oscillations. It is worth noting that we are looking for nonlinear cycles when density dependence gets stronger than threshold, i.e., when $f < 0$. This will happen only if $a_1 < 0$ in (10), in which case the solution (9) is also stable to small perturbations of biomass. While this condition does hold for the Schnute function, it is possible that $a_1 > 0$ for a different nonlinear recruitment model, in which case there will be no sustained oscillations beyond threshold (see Hastings and Costantino (1987) for a biological example of nonlinearity that does not support stable oscillations above threshold).

Our first results concern the coefficients in (10) and (11). In Appendix B we present the explicit analytical expres-

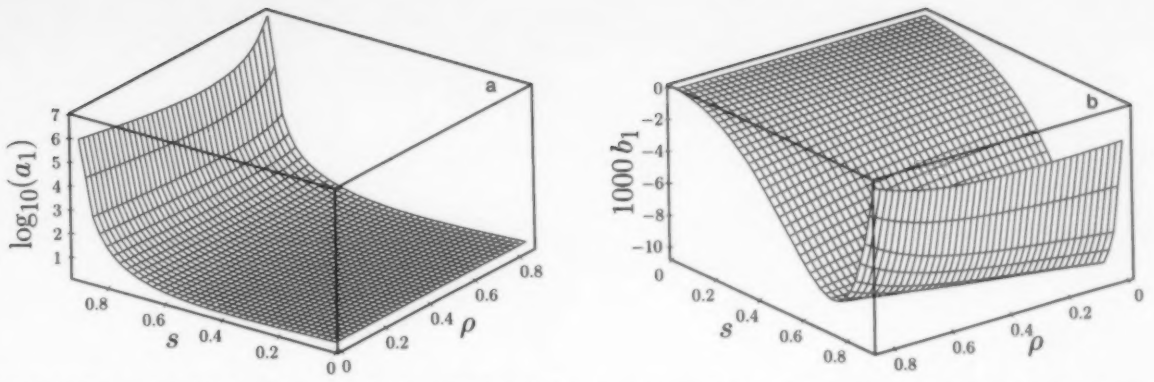

FIG. 2. (a) Perspective plot showing values of $\log_{10}a_1$ for changing ρ and s, with $\tau = 3$, $\gamma = -0.1$, and $V = 0$. (b) Perspective plot showing values of $10^3 b_1$ for changing ρ and s, with $\tau = 3$, $\gamma = -0.1$, and $V = 0$.

sions for these coefficients in terms of the model parameters. We will not dwell on the details of the computation; the formulae for this case are derived from those in Tuljapurkar (1987). Numerical evaluation of these expressions, using the Schnute function, for a range of parameters (not an exhaustive survey, but extensive) shows that b_1 is of the order 10^{-2} to 10^{-3} and is thus very small for the Deriso–Schnute model. The period of the linear model at threshold is thus a very good predictor of the period of nonlinear oscillations beyond threshold. This finding echoes the results of Wachter (1991b) for a nonlinear renewal model of human fertility. We believe that it is a general feature of nonlinear renewal equation models.

The coefficient a_1 is typically much larger than b_1, by one to three orders of magnitude. This means that the increase in density dependence required to produce biomass oscillation of even small amplitude can be very substantial. The two panels of Fig. 2 illustrate the difference between the parameters a_1 and b_1 for various values of the demographic parameters.

What happens as density dependence becomes stronger, so that f is large? A partial answer is given by extending the bifurcation calculations. An extension of the bifurcation analysis, whose details will be given elsewhere (they are available from the authors on request), yields the relationships

$$f = a_1 A^2 + a_2 A^4 + O(A^6) \tag{12}$$

and the period in the form

$$\left(\frac{2\pi}{T}\right) = \left(\frac{2\pi}{T_0}\right) + b_1 A^2 + b_2 A^4 + O(A^6). \tag{13}$$

Numerical evaluation of these coefficients shows that b_2 is of the same order as b_1, reinforcing the conclusion that the period of the cycles changes very little as their amplitude gets large. The coefficient a_2 has the same sign as a_1, and similar magnitude. Thus the more detailed analysis again shows that it takes substantial change in F_1 to generate large-amplitude oscillations.

To illustrate the accuracy of the bifurcation expansions, we show in Fig. 3 two cases where we compare the expansions with the results of numerical iterations of the full nonlinear model (1). In the first case (Fig. 3a), the expansion (10) and (11) is accurate for amplitudes of oscillation as large as 1 of our scaled biomass units. To make sense of this, note that the equilibrium biomass B^* at threshold is 4.4 scaled units, so the largest amplitude shown is about 22% of the equilibrium. In the second case, (Fig. 3b), the expansion (10) and (11) is only accurate for amplitudes up to about 15% of equilibrium biomass B^*, while the more accurate expansion (12) and (13) is accurate for amplitudes up to 45% of the equilibrium biomass.

2.6. Effects of Fishing

We have so far ignored the important question of how fishing mortality affects the dynamics. We describe the effects of fishing by introducing a parameter ϕ equal to the survival probability of a fish from fishing mortality in a year. Schnute (1985) related this probability to fishing effort and also wrote out the formal difference equations for the fishery when effort varies over time. We examine only the case where effort, and thus ϕ, are constant from year to year. The basic renewal equation (1) can now be modified in terms of the unexploited biomass $K_t = \phi B_t$. Renewal involves only this unexploited biomass, so equation (1) yields

$$K_t = \sum_{a \geq 0} \phi^a h_a \, \phi F(K_{t-\tau-a}). \tag{14}$$

Notice that (14) has the same form as equation (1). Hence the nonlinearity in equation (14) is exactly that in equation (1), except that the renewal function in (14) is multiplied by the factor ϕ. In the case of the Deriso–Schnute renewal function, it follows that the dynamics of K_t for given values of ϕ, s, and α are the same as those of B_t but with survival rate in (1) set to $(s\phi)$ and the renewal parameter in (2) set to $(\phi\alpha)$. The dynamic behaviors we describe for model (1) without fishing mortality will all be observed for the uncaught biomass K_t but at shifted parameter values. The extent of the shift depends on the fishing effort via the value of ϕ. The dynamics of the catch are simply those of $[(1 - \phi) K_t/\phi]$.

The qualitative consequences of fishing mortality deserve more discussion. First, increases in fishing reduce ϕ and therefore reduce the overall survival rate of fish. The influence function h_a is then skewed towards earlier ages and also falls off more rapidly at later ages. The typical effect of moderate fishing pressure on the influence function is a decrease in the mean, a faster decrease in the variance, and also a decrease in the sum H. From equation (8) (and some numerical experiments), we conclude that with mod-

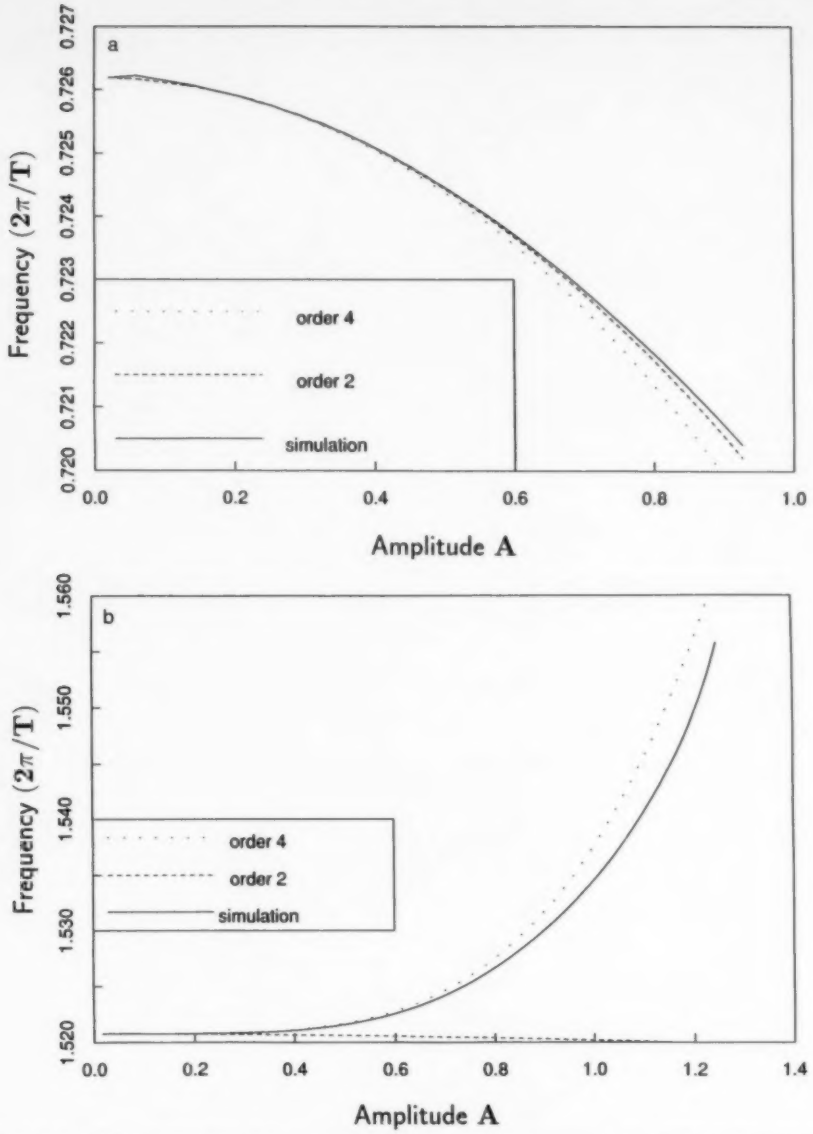

FIG. 3. Predicted versus true (i.e., numerically computed) frequency of oscillation using expansion to second order and to fourth order in amplitude. Parameters are (a) $\tau = 3$, $\gamma = -0.1$, $V = 0$, $\rho = 0.6$, and $s = 0.61$ and (b) $\tau = 2$, $\gamma = -0.1$, and $V = 0$, $\rho = 0.6$, and $s = 0.61$.

erate fishing pressure the threshold magnitude $|\hat{F}_1|$ of response needed for linear instability will increase. On the other hand, the recruitment function in the presence of fishing is multiplied by ϕ, so that the actual slope F_1 that is achieved in a fishery decreases in the presence of fishing mortality. Thus the instability threshold is both higher and harder to reach when there is fishing pressure. This conclusion matches that of Botsford and Wickham (1978) in their analysis of a crab fishery. Second, extreme fishing pressure may push the effective survival probability of fish towards zero, thus reducing the averaging over past cohorts in (14). Such an effect would tend to counteract the stabilizing effect of a reduced slope in the recruitment function. Finally, the dynamic consequences of fishing pressure may be very different in cases where the catch rate at a given level of effort increases with the available biomass. In that case the survival rate ϕ would itself be a decreasing function of the available biomass, so that the nonlinearity in equation (14) would be qualitatively different from that in equation (1). Such a difference may produce qualitatively different dynamics.

2.7. Conclusions of the Nonlinear Analysis

It is not always possible for model (1) to reach a linear stability threshold; the Schnute recruitment function does not always allow it. The "local" shape of the stock–recruitment function near equilibrium determines the oscillations that

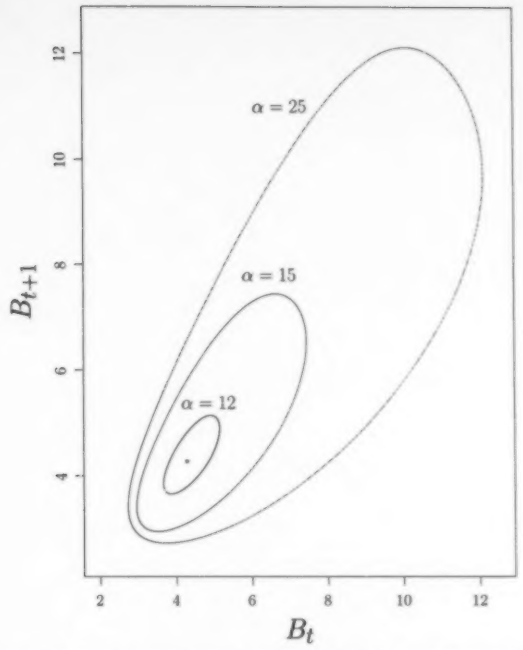

FIG. 4. Dynamic attractors of Schnute model for different fixed α. In every case, τ = 3, γ = −0.1, and V = 0.6, ρ = 0.6, and s = 0.6; at the fixed point, α is 11.5 and increases through values 12, 15, and 25 as we move outward.

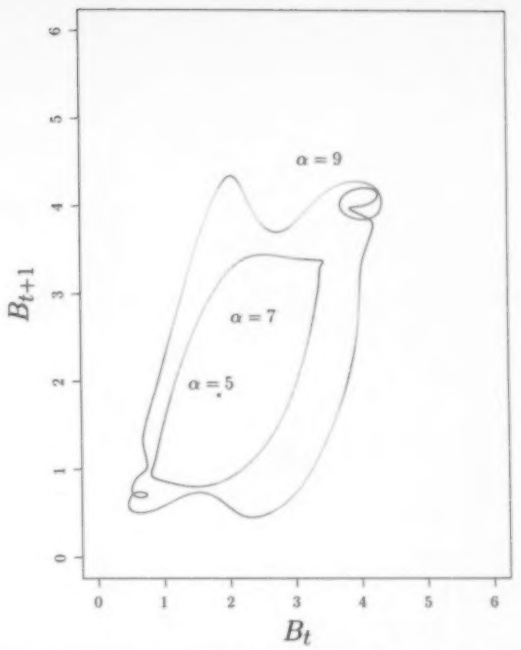

FIG. 5. First steps toward chaotic behavior of Schnute model for different fixed α. In every case, τ = 4, γ = 0.05, V = 0, ρ = 0.4, and s = 0.21; at the fixed point, α is 5 and increases through values 7 and 9.

the nonlinear model can support. The period of nonlinear oscillations is always very close to that obtained from the linear model at threshold. But the amplitude of oscillations responds very slowly to increases in the magnitude $|F_1|$ of the slope. It takes a considerable change in this slope to generate cycles whose amplitude is more than a few percent of equilibrium biomass. Typically, recruitment has to be much more sensitive to density than it is at threshold for large oscillations to be sustained. We will return to these conclusions when we consider the relationship of our results to empirical work.

3. Very Strong Density Dependence

In the preceding section, we considered the effect of increasing density response $|F_1|$ of recruitment at equilibrium. In theory, it is always possible to set this response to values far greater than the threshold magnitude $|\hat{F}_1|$. When recruitment is governed by a global function, such as Schnute's, large changes in the slope at equilibrium also produce large changes in the slope at small biomass values. The result is that the biomass can vary substantially from year to year. There is a burgeoning literature on the dynamics of nonlinear equations that contain very strong density dependence (for a recent contribution with useful references, see Hastings et al. 1993).

What is the effect of strong density dependence on model (1)? Our bifurcation analysis shows that as F_1 increases, biomass first becomes linearly unstable around B^* and then sustained oscillations occur. The amplitude of these oscillations increases (albeit slowly) with F_1. Further increase in F_1 can, in principle, lead to two kinds of dynamics. One possibility is that the oscillation persists at increasing amplitude, but with complex periodicity. Another is that the oscillation starts to become increasingly irregular (aperiodic or multiply periodic) and then the oscillatory pattern is replaced by increasingly complex time-varying patterns, possibly chaotic. The techniques we used to analyze feedback cycles have no adequate counterpart for more complex dynamics; analytical techniques for these phenomena have to be used in a diagnostic fashion in conjunction with numerical iterations of a model.

For the Deriso–Schnute model, we used numerical iterations to explore the effects of very strong density dependence. We did this by choosing parameter sets and then increasing α in the Schnute function. We find that over a wide range of the parameters, the model displays oscillations that resemble those found by the bifurcation analysis. While the period of the oscillations can vary a great deal, and the amplitude increases, the trajectory of biomass retains its shape. The term "shape" here refers to the appearance of a plot of a trajectory of the model in a plane whose coordinates are B_{t+1} and B_t. See Fig. 4 for a typical example: the successively larger closed curves shown are stable attracting trajectories of biomass corresponding to successively higher fixed values of α in the Schnute recruitment function. The dot interior to all the closed curves is the fixed equilibrium that occurs for relatively low α; parameter values are given in the legend to the figure.

However, when we consider small values of both s and ρ, together with positive values of γ and small values of τ (usually 3 or 4), we do find that the cyclical dynamics are replaced by complex dynamics. In one example of this (Fig. 5), the stable fixed equilibrium for α = 5 is replaced by a stable cycle for α = 7. When α is set to a higher value

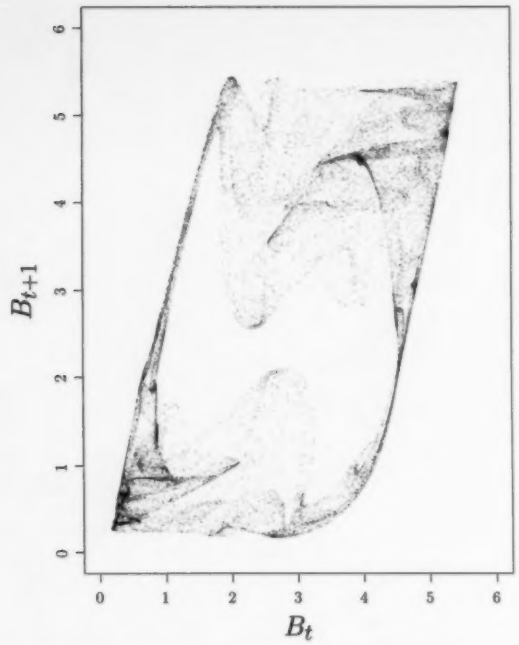

FIG. 6. Apparent chaotic behavior of Schnute model for $\alpha = 11$ and other parameters as for Fig. 5.

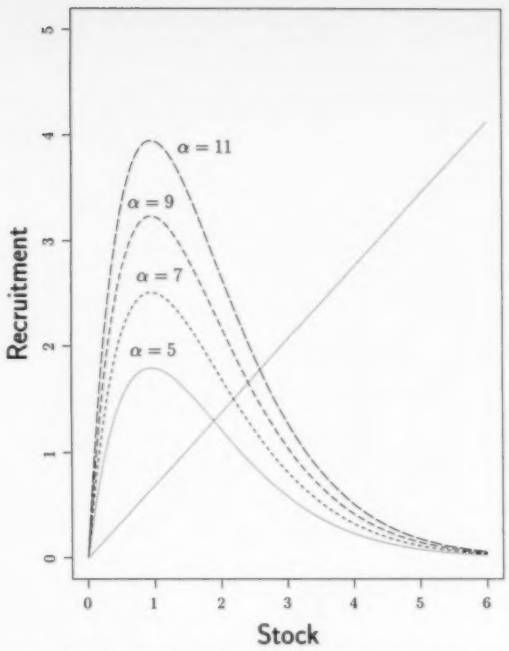

Fig. 7. Schnute stock–recruitment function (in scaled units), showing equilibrium biomass values, for the parameter values used in Figs. 5 and 6 and with the parameter α as indicated. The line intersects the curves at equilibrium.

of 9, an expanded cyclical attractor is found but it displays "folds" at some points. Setting $\alpha = 11$ results in what is most likely a chaotic attractor (Fig. 6).

Finding complex attractors in this model took some work. Our experience shows, contrary to conventional wisdom, that a high-dimensional nonlinear model (of which (1) is an example) may display chaos for only a limited set of parameter choices. Furthermore, complex dynamics require that recruitment be extremely sensitive to changes in stock level over a wide range of stocks. Figure 7 shows the shape of the Schnute function corresponding to the successive attractors in Fig. 6. Compare the lower two curves (which yield a point equilibrium or a limit cycle) with the upper two (which yield complicated attractors), especially in terms of the slope near zero, the maximum level of recruitment, and the slope near maximum recruitment. To generate chaos requires a tremendous responsiveness of recruitment to stock that is biologically implausible, and we are compelled to the view that chaotic dynamics in fish populations are unlikely to be generated in this manner. (This does not rule out a different underlying mechanism that might produce chaos.)

Our final observation about complex dynamics is that they are generated by globally strong density dependence. This point has been noted in the theory of nonlinear difference equations (Guckenheimer and Holmes 1983), where it is known that one source of chaos is to have strong local instability (say at equilibrium) coupled with large density response at very different population size (or biomass). It has also been noted by Botsford (1992) in an analysis of his model for Dungeness crab, where he found that complex aperiodic dynamics occurred only when recruitment was extremely sensitive to density over a wide range.

4. Local versus Global Density Dependence

Stock–recruitment relationships such as Schnute's have a long tradition in fisheries analysis. As Ricker pointed out in 1954, they have a sound logical basis. But they have also been notoriously difficult to estimate. Walters and Ludwig (1981) pointed out difficulties caused by noise in the data, Cushing's (1971) survey pointed to the inadequacy of data over a wide biomass range, and recently, Fogarty et al. (1991) and Sheperd and Cushing (1990) have questioned the use of such functions to capture the biology of density-dependent interactions. In practice, it is likely that we can only estimate a stock–recruitment relationship over a limited range of stock levels.

These factors lead us to argue that a "global" stock–recruitment relationship may not be the best or the only way to think about density dependence. Rather, we may use "local" relationships. Figure 8 illustrates the local view and is constructed by using the Schnute function at low stocks and near equilibrium; between the two regions, we assume that recruitment is independent of density. The behavior near zero and near equilibrium is consistent with any number of functions that "locally" have the same behavior as Schnute's function. At low stocks, we can use appropriate estimates of α; near equilibrium, we can use estimates of the local density response F_1. At intermediate stock levels, Fig. 8 makes the neutral hypothesis that stock has little effect on recruitment. Patching together local density dependence in this way is one version of Strong's (1984) "density-vague" regulation.

What are the dynamic consequences of a local approach to density effects? Figure 9 shows the pattern of biomass

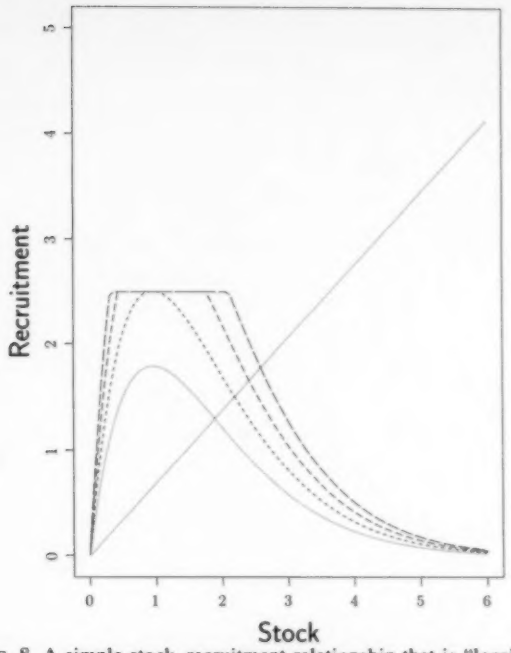

FIG. 8. A simple stock–recruitment relationship that is "locally" defined at low and near-equilibrium stock levels and has bounded response to stock levels. Parameter values used are those in Fig. 7. Recruitment is bounded at 2.5 units.

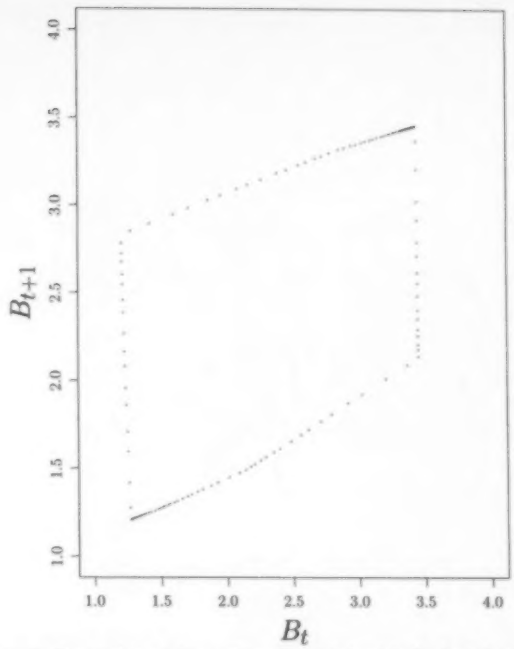

Fig. 9. Dynamics under a "local" model with limited density response. Parameters correspond to those for Fig. 6, except that the stock–recruitment curve is bounded as in Fig. 8.

dynamics for model (1) using the local recruitment function of Fig. 8. The response of recruitment to stock "locally," near equilibrium, is sufficient to generate sustained oscillations of the sort we have analyzed here. But locally strong density dependence does not lead to the complex dynamics that appear in Fig. 6 for the "global" Schnute function.

The nonlinear analysis we presented in Section 2 is applicable to a local function just as it is to the Schnute function, so we can use those results in either case.

5. Conclusions

Our examination of the nonlinear forces that maintain sustained biomass oscillations in the Deriso–Schnute model leads to conclusions relevant to modeling and data analysis.

First, consider the analysis of sustained oscillations that are observed in a fishery. The key characteristics of any hypothesized model of the type (1) are that the period of oscillations changes very slowly with the level of density dependence, and it takes substantial changes in density dependence to produce changes in the amplitude of oscillation. Therefore, if we observe the same fish species in two regions, we can compare the period and amplitude of oscillation. We expect the periods to be very similar even if the amplitudes differ. In estimating the model, we may proceed by matching parameters to generate the observed period and then predict the amplitude. Because we have shown that amplitude and period respond so differently to density dependence, this method is capable of providing a sensitive test of a model. (See Wachter (1991b) for an example of such a test applied to human fertility data.)

Second, the dynamics of models with strong density dependence are sensitive to the global character of the density response. Unless we are confident of our estimation of response, we may do well to start with a locally defined pattern of density dependence. The analytical methods we present here generate useful information about the behavior of such locally specified models.

Third, the dynamics we have described in the absence of fishing are also relevant in the presence of fishing. Fishing mortality may be described by considering the unexploited fraction of the biomass, the dynamics of which are essentially those of an unfished stock but with reduced mortality and recruitment.

Acknowledgements

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Appendix A. Linear Stability Threshold

The stability threshold is defined by the condition $|\lambda| = 1$, which means that $\lambda = \exp(i\omega_0)$ where $\omega_0 = (2\pi/T_0)$. The linear stability equation (5) with $x_t = \lambda^t$ can be written as

$$\sum_a (h_a / H) \exp(-ia\omega_0) = \frac{1}{HF_1}.$$

Taking the logarithm of both sides yields a cumulant generating function on the left; expand this and then use the method of reversion of series to get (8). Demographers have long used this expansion method (LeBras 1969; Frauenthal 1975). Keyfitz (1985) gave a detailed application of it, and this paper follows an analysis for a human example by Wachter (1991b), who presented further references and much more detail.

Appendix B. Nonlinear Cycles

The bifurcation expansions in (10) and (11) are derived using the methods in Staffans (1987) and Tuljapurkar (1987). The calculation is quicker if we follow Schnute (1985) and rewrite the nonlinear model (1) as

$$B_t = (1+\rho)sB_{t-1} - \rho s^2 B_{t-2} + R_t - \rho s V R_{t-1}.$$

Setting $B_t = B^* + x_t$ as in the text and expanding as in (4) yields the linear term $L_t(x)$ (which we do not write out) and nonlinear terms of order $l \geq 2$ that have the form

$$Q_l(x_t) = F_l[\, x_{t-\tau}^l - \rho s V x_{t-\tau-1}^l \,]$$

where F_l is the product of $(1/l!)$ and the lth derivative of the recruitment function R evaluated at equilibrium.

The bifurcation expansion uses the threshold solution as a starting point. Recall from Appendix A that ω_0 is the frequency at threshold, and write $\hat{F}_l$ for $l \geq 1$ for the coefficients evaluated at threshold. Define

$$z = e^{i w_0}, \text{ with } i = \sqrt{(-1)},$$

and for any complex y define

$$\Delta(y) = 1 - [(1+\rho)sy^{-1} - \rho s^2 y^{-2} + \hat{F}_1 y^{-\tau}(1 - \rho s V y^{-1})]$$

and

$$W_l(y) = \hat{F}_l y^{-l\tau}[1 - \rho s V y^{-1}].$$

Using these objects, compute the complex quantities

$$K = \frac{4W_2(1)W_2(z)}{\Delta(1)} + \frac{2W_2(z^2)W_2(z)}{\Delta(z^2)} + 3W_3(z)$$

$$\Delta_1 = [W_1(z)]/\hat{F}_1$$

$$\Delta_2 = iz\left(\frac{d\Delta(z)}{dz}\right).$$

Then the desired coefficients a_1 and b_1 are real numbers

solving the equation (with complex coefficients; take real and imaginary parts to solve)

$$a_1\Delta_1 + b_1\Delta_2 = K.$$

To use these formulae for the Schnute function, it is necessary to compute the equilibrium and threshold values plus the derivatives.

A Nomenclatural Review of *Dreissena* (Bivalvia: Dreissenidae), with Identification of the Quagga Mussel as *Dreissena bugensis*

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Rosenberg, G., and M.L. Ludyanskiy. 1994. A nomenclatural review of *Dreissena* (Bivalvia: Dreissenidae), with identification of the quagga mussel as *Dreissena bugensis*. Can. J. Fish. Aquat. Sci. 51: 1474–1484.

A review of systematic literature and examination of type material of *Dreissena* (Paleontological Institute, Moscow) revealed that the quagga mussel corresponds to the original description and type specimens of *Dreissena bugensis* Andrusov, 1897. Russian classifications have treated *D. bugensis* as a full species or as a subspecies of *D. rostriformis* Deshayes, 1838. *Dreissena rostriformis* is the type species of the subgenus *Pontodreissena*. *Dreissena rostriformis rostriformis* is regarded as extinct, with two lineages derived from it still living: *Dreissena bugensis*, in the Dnieper and Bug rivers and the estuary where they enter the Black Sea, and *D. rostriformis grimmi*, in the southern Caspian Sea. These lineages differ in morphology, coloration, maximum adult size, and salinity tolerance and are therefore regarded as separate species. We adopt the name *Dreissena (Pontodreissena) bugensis* for the quagga mussel and designate and illustrate lectotypes for *D. bugensis* and other dreissenid taxa named by Andrusov. Including *D. rostriformis* and *D. bugensis*, seven living species of *Dreissena* are currently recognized. The nomenclatural histories of these species are summarized and the supraspecific classification of dreissenids is reviewed.

Un examen des publications taxonomiques et une étude du matériel type de *Dreissena* (Institut de paléontologie de Moscou) a révélé que la moule quagga correspond à la description initiale et aux spécimens types de *Dreissena bugensis* Andrusov, 1897. Les classifications russes ont traité *D. bugensis* comme une espèce à part entière ou comme une sous-espèce de *D. rostriformis* Deshayes, 1838. *Dreissena rostriformis* est l'espèce type du sous-genre *Pontodreissena*. *Dreissena rostriformis rostriformis* est considérée comme étant éteinte, mais deux lignées dérivées vivent toujours : *Dreissena bugensis*, dans le Dnieper et le Bug ainsi que dans l'estuaire où elles pénètrent dans la mer Noire, et *D. rostriformis grimmi*, dans la partie méridionale de la mer Caspienne. Ces lignées diffèrent sur le plan de la morphologie, de la couleur, de la taille maximale à l'âge adulte et de la tolérance à la salinité et sont par conséquent considérées comme des espèces distinctes. Nous avons adopté le nom *Dreissena (Pontodreissena) bugensis* pour la moule quagga et nous désignons et illustrons les lectotypes de *D. bugensis* et d'autres taxons de dreissénidés nommés par Andrusov. En comptant *D. rostriformis* et *D. bugensis*, sept espèces vivantes de *Dreissena* sont actuellement reconnues. Nous résumons l'historique de la nomenclature de ces espèces et passons en revue la classification supraspécifique des dreissénidés.

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Interest in the taxonomy of *Dreissena* has surged following the recent introduction into North America of the zebra mussel, *Dreissena polymorpha*, and a second, unidentified *Dreissena* species known as the quagga mussel (Hebert et al. 1989, 1991; Griffiths et al. 1991; May and Marsden 1992; Pathy and Mackie 1993). Identification of the quagga mussel has been problematic because the majority of literature on dreissenid taxonomy is in Russian and has not been readily accessible to Western researchers. Also, some Western studies of dreissenids, for example, Archambault-Guezou (1976) and Nuttall (1990a), have received little notice from Russian scientists. Russian supraspecific classifications of dreissenids are reasonably concordant with Western ones. Species-level classifications, however, are difficult to compare because there has never been a thorough review of living *Dreissena* species in the Western literature and because different groups of workers have used different criteria for recognizing species and subspecies. Here, we summarize ideas on the taxonomy of dreissenids as reflected in the Russian literature and integrate them with Western ideas in order to determine the correct name for the quagga mussel.

Methods

We attempted to examine all published works in which supraspecific taxa of dreissenids were named or in which taxonomic changes affecting living species of *Dreissena* were made. We located such works by reference to the *Zoological Record* and the bibliographies of major taxonomic studies of *Dreissena*. We have taken a historical approach, tracing changes in dreissenid taxonomy over time and the justifications for those changes, particularly at the species level.

TABLE 1. Supraspecific classifications of Dreissenidae. Generic names are in boldface, subgeneric names are in parentheses, and informal group names are in quotation marks; "+" precedes names in synonymy. Andrusov (1897) used the spellings *Dreissensia* and *Dreissensiomya*, both of which are unjustified emendations.

Andrusov (1897)	Starobogatov (1970)	Babak (1983)	Nuttall (1990a)	Herein
Dreissenidae	Dreissenidae	Dreissenidae	Dreissenidae	Dreissenidae
—	—	Dreisseninae	Dreisseninae	Dreisseninae
Dreissensia	***Dreissena***	***Dreissena***	***Dreissena***	***Dreissena***
"carinatae"	(*Dreissena*)	(*Dreissena*)	(*Dreissena*)	(*Dreissena*)
—	—	—	—	(*Carinodreissena*)
—	—	(*Modiolodreissena*)	(*Modiolodreissena*)	(*Modiolodreissena*)
"rostriformes"	(*Pontodreissena*)	(*Pontodreissena*)	(*Pontodreissena*)	(*Pontodreissena*)
Congeria	***Congeria***	***Congeria***	***Congeria***	***Congeria***
"subglobosae"	(*Congeria*)	(*Congeria*)	—	(*Congeria*)
—	—	+ *Globocongeria*	+ *Globocongeria*	+ *Globocongeria*
"rhomboideae"	(*Rhombocongeria*)	(*Rhombocongeria*)	+ *Rhombocongeria*	+ *Rhombocongeria*
—	—	+ *Rhomboidea*	+ *Rhomboide*	+ *Rhomboidea*
—	—	—	—	(*Filicarina*)
"mytiliformes"	(*Mytilopsis*)	(*Mytilopsis*)	***Mytilopsis***	***Mytilopsis***
—	—	+ *Mytilia*	+ *Mytilia*	+ *Mytilia*
"eocaenae"	(*Eocongeria*)	(*Eocongeria*)	+ *Eocongeria*	+ *Eocongeria*
"modioliformes"	(*Andrusoviconcha*)	(*Andrusoviconcha*)	+ *Andrusoviconcha*	+ *Andrusoviconcha*
—	—	+ *Modiola*	+ *Modiola*	+ *Modiola*
"triangulares"	(*Trigonipraxis*)	(*Trigonipraxis*)	+ *Trigonipraxis*	+ *Trigonipraxis*
—	—	+ *Triangularia*	+ *Triangularia*	+ *Triangularia*
—	—	—	**Prodreissensia**	**Prodreissensia**
—	—	Dreissenomyinae	Dreissenomyinae	Dreissenomyinae
Dreissensiomya	***Dreissenomya***	***Dreissenomya***	***Dreissenomya***	***Dreissenomya***
—	—	(*Dreissenomya*)	(*Dreissenomya*)	(*Dreissenomya*)
—	—	(*Sinucongeria*)	(*Sinucongeria*)	(*Sinucongeria*)

Our taxonomic decisions were guided by study of Andrusov's collection of Dreissenidae at the Paleontological Institute in Moscow (PIM) and Logvinenko's Caspian *Dreissena* at the Zoological Institute in St. Petersburg in August and September 1993. We also collected and observed living *Dreissena* in the Dnieper River and Liman (estuary) in the Ukraine and the Volga Drainage in Russia; these specimens are deposited at the Academy of Natural Sciences of Philadelphia (ANSP).

Early Studies of Dreissenids

Dreissena polymorpha (Pallas, 1771) was first found in a tributary of the Ural River on 12 August 1769 by the famous Russian scientist and explorer Pyotr Simon Pallas (1741–1811). He classified it as a *Mytilus* because of the external resemblance of the shell to members of that exclusively marine genus. Pallas (1771) stated that he knew of the species only from the Volga and Yaik (= Ural) rivers and the Caspian Sea. van Beneden (1835) named the genus *Dreissena*, with type species *Mytilus polymorphus* Pallas, distinguishing it from *Mytilus* on the basis of anatomy and internal shell morphology. A year later, Partsch (1836) described the genus *Congeria* from Miocene deposits of the Vienna basin, noting the similarity of the shells of *Congeria* and *Dreissena*. In 1840, Gray named the family Dreissenidae, including *Congeria* and *Mytilimeria* Conrad, 1837; the latter is now placed in the Lyonsiidae (Vokes 1980). Other genera, such as *Mytilopsis* Conrad, 1858, named for living New World species, *Dreissenomya* Fuchs, 1870 and *Sinucongeria* Lörenthey, 1894, were known to be dreissenids when they were named. By the 1850s, it was well-known that *Dreissena* bore only a superficial resemblance to *Mytilus*, and Gill (1871) classified Dreissenidae in a different order from Mytilidae, placing it in the superfamily Dreissenacea next to Corbiculacea and Veneracea, essentially the same position as in modern classifications.

One of the first workers to review the classification of Dreissenidae was Oppenheim (1891), but his studies were soon eclipsed by the monumental researches of Andrusov, the first Russian scientist to work extensively on *Dreissena* (1890, 1893, 1897, 1900). Andrusov's *magnum opus* (1897) was his 700-page monograph of living and fossil *Dreissena*, in which he summarized previous information on all known species, named many new taxa, and diagrammed hypothesized relationships among lineages through the fossil record to the Recent fauna.

Supraspecific Classification

Supraspecific classifications of Dreissena are summarized in Table 1. Andrusov (1897) divided *Dreissena* into two species-groups, *carinatae* and *rostriformes*, and *Congeria* into six species-groups; his classification survived unchanged for almost 70 yr until Logvinenko and Starobogatov (1966) named the subgenus *Pontodreissena*, corresponding to Andrusov's *rostriformes*. The prominent diagonal ridge of *Dreissena (Dreissena)* is absent or mainly confined to the umbonal region in *Pontodreissena* (Nuttall 1990a). Starobogatov (1970) reclassified Andrusov's species-groups of *Congeria* as subgenera, naming *Andrusoviconcha, Eocongeria, Rhombocongeria,* and *Trigonipraxis*. He thereby established the basic classification of dreissenids used by subsequent Russian workers. Marinescu (1973) independently named some of these species-groups *Modiola, Mytilia, Rhomboidea,* and *Triangularia,* unaware that they had already been named (see Nuttall 1990a). Since Starobogatov (1970) proposed his classification, several changes have occurred in

TABLE 2. Classification of living species of *Dreissena*.

Andrusov (1897)[a]	Zhadin (1952)	Mordukhai-Boltovskoi (1960)[b]	Logvinenko (1965)[c]	Logvinenko and Starobogatov (1968)
Dreissena	*Dreissena*	*Dreissena*	*Dreissena*	*D. (Dreissena)*
polymorpha	*polymorpha*	*polymorpha*	*polymorpha*	*polymorpha*
v. *polymorpha*	v. *polymorpha*	*p.* subspecies	*p. polymorpha*	*p. polymorpha*
		p. subspecies	*p. andrusovi*	*p. andrusovi*
v. *aralensis*	v. *aralensis*	—	—	*p. aralensis*
v. *obtusecarinata*	v. *obtusecarinata*	—	—	*p. obtusecarinata*
v. *elata*	—	*elata*	*elata*	*elata*
andrusovi	*andrusovi*			
caspia	*caspia*	*caspia*	*caspia*	*caspia*
				c. caspia
pallasi	*pallasi*	—	—	*c. pallasi*
bourguignati[e]	—	—	—	—
				D. (Pontodreissena)
rostriformis	—	*rostriformis*	*rostriformis*	*rostriformis*
v. *distincta*	*distincta*	*r.* subspecies	*r. distincta*	*r. distincta*
grimmi	*grimmi*	*r.* subspecies	*r. grimmi*	*r. grimmi*
		r. subspecies	*r. pontocaspica*[f]	*r. pontocaspica*
		r. subspecies	*r. n. sbsp.*	*r. compressa*
bugensis	*bugensis*	*r. bugensis*	—	—

[a]Only those varieties recognized by other authors in Table 1 are listed.
[b]Mordukhai-Boltovskoi specified the number of subspecies in the Caspian but did not state their names.
[c]Logvinenko (1965) treated only forms from the Caspian.
[d]Starobogatov has completed a revision of living and fossil Dreissenidae, but the manuscript is delayed in press.
[e]The status of *D. bourguignati*, *D. diluvii*, and *D. caputlacus* as synonyms or separate species is uncertain.
[f]Named as a fossil by Andrusov, who did not recognize it as a living variety.

the Russian literature: (1) two subgenera of *Dreissena* have been named, *Carinodreissena* L'vova & Starobogatov, 1982 and *Modiolodreissena* Babak, 1983, the latter being extinct, and (2) Taktakishvili (1973) created the subfamily Dreissenomyinae for *Dreissenomya* and *Sinucongeria*. The most recently published Russian classification of dreissenids, by Babak (1983), incorporated these changes, except that *Carinodreissena* was omitted, having been named too late for inclusion.

The most important difference between Russian and Western treatments of dreissenids is that the Russians classify *Mytilopsis* as a subgenus of *Congeria* whereas Western workers since Keen (1969) and early as Maury (1917) classify it as a full genus (Nuttall 1990a, 1990b). Nuttall (1990a) synonymized *Eocongeria, Trigonipraxis,* and *Andrusoviconcha* with *Mytilopsis;* Starobogatov (1970) had named these as subgenera of *Congeria.* Few other changes in dreissenid taxonomy have been proposed in non-Russian sources since Starobogatov's (1970) classification. Marinescu (1992) named *Filicarina* as a subgenus of *Congeria* and Nuttall (1990a), following Keen (1969), tentatively included in Dreissenidae *Prodreissensia* Rovereto, 1898, a problematic fossil from the Lower Oligocene of Italy. Nuttall (1990a) did not treat *Carinodreissena* in his revision of the Dreissenidae, presumably an oversight. None of the recent changes in dreissenid taxonomy affect the genus *Dreissena* itself, so the most recent Russian and Western classifications of *Dreissena* are concordant.

All classifications of Dreissenidae to date have been based on phenetic (overall similarity) or stratophenetic methods (overall similarity taking into account stratigraphic position); classifications produced by cladistic methods (derived similarity) will likely differ (see Allmon 1989). We therefore do not revise the classification of Dreissenidae, but merely update the most recent classification (Nuttall 1990a) by adding the taxa not treated therein (*Carinodreissena* and *Filicarina*) (see Table 1).

Species-level Classification

Although many studies of *Dreissena* have dealt with both living and fossil species, in summarizing the literature we have concentrated on living species because establishing their identities is most critical to investigations of *Dreissena* introduced into North America. Dreissenids are highly variable in shell morphology and coloration, and more than 150 names have been introduced for Recent and fossil *Dreissena.* Babak (1983) has noted that many taxa have been distinguished without taking into account intraspecific variability, which has led to the naming of many so-called "morphological" species.

Locard (1893) recognized 30 species of *Dreissena* in Europe and Asia Minor, more than any other author, but most of these have since been synonymized with *Dreissena polymorpha* by Andrusov (1897), Kennard and Woodward (1926), and Germain (1931). Russian workers have recognized as many as seven or eight living species of *Dreissena* (Andrusov 1897; Zhadin 1952) whereas Western workers often have assumed that there are only one or two. For example, Marelli and Gray (1985) stated that *Dreissena* is "represented by a single species restricted to Great Britain and continental Europe." Nuttall (1990a) mentioned only two living *Dreissena* species, *D. (Dreissena) polymorpha* and *D. (Pontodreissena) rostriformis*, but he treated mainly

Starobogatov (MS)[d]	Herein
D. (Dreissena)	*D. (Dreissena)*
polymorpha	*polymorpha*
p. polymorpha	*p. polymorpha*
p. andrusovi	*+ andrusovi*
p. aralensis	*p. aralensis*
p. obtusecarinata	*p. obtusecarinata*
elata	*elata*
andrusovi	*andrusovi*
caspia	*caspia*
c. caspia	*c. caspia*
c. pallasi	*c. pallasi*
diluvii[e]	*caputlacus*[e]
D. (Carinodreissena)	*D. (Carinodreissena)*
stankovici	*stankovici*
D. (Pontodreissena)	*D. (Pontodreissena)*
rostriformis	*rostriformis*
r. distincta	*r. grimmi*
r. grimmi	*+ distincta*
r. pontocaspica	*+ pontocaspica*
r. compressa	*+ compressa*
bugensis	*bugensis*

the higher classification of dreissenids and did not claim to be exhaustive for species-level taxa.

Living Species of *Dreissena*

Classifications of living species of *Dreissena* are summarized in Table 2. Andrusov (1897) recognized eight living *Dreissena* species in Eurasia. Zhadin (1952) recognized seven of these species and reproduced some of Andrusov's illustrations; however, his illustration of *D. bugensis* is incorrect, as discussed below. Zhadin's classification is almost identical to Andrusov's except that *D. bourguignati* Locard, 1883 is excluded (not occurring in the Soviet Union) and *D. distincta* rather than *D. rostriformis* is recognized as a living species. Zhadin's (1952) classification is now outdated, but it is the only Russian systematic work treating *Dreissena* that has been translated into English, and it is therefore the only one usually consulted by Western workers.

The basis for current Russian taxonomy of *Dreissena* was first suggested by Mordukhai-Boltovskoi (1960), who recognized four species in the Caspian Sea, *D. polymorpha*, *D. elata*, *D. caspia*, and *D. rostriformis*, including two unspecified subspecies of *D. polymorpha* and four of *rostriformis*. This classification was confirmed by Logvinenko (1965), who performed extensive morphological studies of Caspian *Dreissena*. Logvinenko used explicit criteria for determining species boundaries in *Dreissena*: independent species are separated by morphological gaps whereas subspecies grade continuously in morphology across ecological gradients. Subsequent Russian studies have recognized the same four species in the former Soviet Union, with varying number of subspecies, but *D. bugensis* has recently been restored to species status (Table 2). Only two other living *Dreissena* species are generally recognized, *D. stankovici* L'vova & Starobogatov, 1982 from Lake Ohrid (on the border of Albania and Macedonia) and *D. caputlacus* Schütt, 1993, named from Turkey. *Dreissena* species formerly reported from Southeast Asia are now classified as *Sinomytilus* (Mytilidae). The status of each of the seven living species of *Dreissena* is discussed below.

Dreissena rostriformis *Deshayes, 1838 (Fig. 1f, 2)*

Dreissena rostriformis is quite variable in morphology and has been subdivided into a number of varieties and subspecies, some of which have also been treated as full species (Table 2). There is considerable debate as to whether the name *Dreissena rostriformis*, proposed for Pliocene fossils from the Crimea (Fig. 1f, 2a), is applicable to the living fauna or represents the extinct ancestor of the living forms. For example, Nuttall (1990a) considered *D. rostriformis* to be a long-ranging species that originated in the Late Miocene (about 7 million yr ago) and persists to the present day whereas Babak (1983) considered *D. rostriformis rostriformis* to have gone extinct in the Upper Pliocene and *D. rostriformis distincta* to be the ancestor of *D. rostriformis bugensis*.

Dreissena rostriformis was widespread when the Caspian, Black, and Aral seas formed a continuous body of water, with a range extending from the Caspian to as far west as Croatia (Basch and Žagar-Sakač 1992). Populations derived from *D. rostriformis* persist in the middle to southern Caspian Sea and in the Dnieper and Bug rivers and the Dnieper/Bug Liman on the north coast of the Black Sea. The Caspian population does not reach the northern Caspian, where salinity is lowered by the flow of the Volga River. We argue below that the two living populations are distinct in morphology and salinity tolerance and should be separated at the species level. However, going back into the fossil record, intermediates can be found, and it is difficult to draw a line between *Dreissena rostriformis rostriformis* and the lineages derived from it. We discuss the Caspian population in this section and the Dnieper/Bug population under *D. bugensis* below.

Logvinenko (1965; Table 2) considered *D. pontocaspica* Andrusov, 1897 (Fig. 2b–2g), *D. distincta* Andrusov, 1897 (Fig. 2h), *D. compressa* Logvinenko & Starobogatov, 1966 (Fig. 2i), and *D. grimmi* Andrusov, 1890 (Fig. 2j) to be subspecies of *D. rostriformis* because after studying many samples, he could not identify morphological gaps separating them. He treated them as essentially sympatric subspecies, with different depth ranges within the Caspian Sea, recognizing that transitional forms existed. Many Western workers, influenced by the biological species concept, would reject the idea of sympatric subspecies, taking geographical isolation as part of the definition of subspecies (Mayr 1976). Logvinenko's work must be understood in the context of Lysenkoism (neo-Lamarckism). Many Russian workers were influenced by Lysenko, who attempted to demonstrate the formation of species in response to environmental changes via the inheritance of acquired (rather than genetic) characteristics. In a Lysenkoist view, sympatric subspecies are expected as part of the process of speciation. Russian and Western definitions of geological subspecies (separated by time rather than space), however, are relatively concordant, both viewing subspecies as being less distinct morphologically than full species (Orlov 1959; Raup and Stanley 1971).

Nevesskaya (1965) considered *D. rostriformis* to be highly polymorphic and included *D. bugensis*, *D. grimmi*, and *D. tschaudae* (Fig. 1g) as synonyms. She recognized two ecological subspecies, *D. r. distincta* occurring in deep water and *D. pontocaspica* in shallow water. She also mentioned *D. r. bugensis* as a subspecies, recognizing that it typically

a
b
c
d
e
f
g
h
i

lived in salinities from 0.2 to 1 ppt whereas the Caspian subspecies lived in higher salinities, up to 12.6 ppt. Babak (1983) recognized a series of 13 intergrading geological subspecies of *D. rostriformis*, with *D. rostriformis bugensis* as the living representative in the Black Sea Basin, but did not treat the living Caspian populations.

Archambault-Guezou (1976) designated a lectotype for *Dreissena rostriformis* (Fig. 2a) and illustrated 16 specimens from its type series. She synonymized most of the forms and subspecies of *D. rostriformis*, including *D. pontocaspica* and *D. distincta*, on the basis of morphometric studies. Unfortunately, she considered only taxa named as fossils and therefore excluded *D. grimmi* and *D. bugensis*. Her morphometric sample also does not seem to have included specimens of the three fossil taxa with the most inflated shells, *D. tschaudae* and *D. rostriformis* vars. *gibba* and *vulgaris*, although these are included in the synonymy.

In summary, all of the forms of *D. rostriformis* in the Caspian Sea intergrade, as demonstrated by the work of Logvinenko (1965), Archambault-Guezou (1976), and Babak (1983). At the Zoological Institute in St. Petersburg, while one of us (G.R.) was examining Logvinenko's Caspian samples of *D. rostriformis*, Dr. Starobogatov confirmed that he often needs to know the depth in which the samples were collected to determine which subspecies name to use. No recent revision based on morphological, paleontological, or ecological evidence has given the Caspian forms of *D. rostriformis* greater than subspecific rank. We conclude that *D. pontocaspica, D. distincta, D. compressa*, and *D. grimmi* are synonyms of *D. rostriformis* (Fig. 2). There may be some justification for maintaining a distinction between an extinct subspecies, *D. rostriformis rostriformis* and a living one, for which *D. rostriformis grimmi* is the oldest name. Fossil specimens of *D. rostriformis* reach a length of at least 37 mm (Archambault-Guezou 1976) whereas living specimens reach only 23 mm (Logvinenko and Starobogatov 1968). Zhadin's (1952) 24-mm record for *D. distincta* is based on a fossil specimen in Andrusov (1897). We designate lectotypes for *D. grimmi* (Fig. 2j) and *D. distincta* (Fig. 2h) to ensure stability of nomenclature.

Dreissena bugensis *Andrusov, 1897 (Fig. 1a–1e)*

Specimens of the quagga mussel introduced into the United States (Fig. 1d and 1e) correspond to the original description and type specimens of *Dreissena bugensis* Andrusov, 1897 (Fig. 1a and 1b). Zhadin's illustration of *D. bugensis* (Fig. 1c), stated to be copied from Andrusov's original, is incorrect, which has complicated efforts to identify the quagga mussel. The specimen in Fig. 1a is designated the lectotype of *D. bugensis* in order to firmly establish the identity of the species. *Dreissena bugensis* can be distinguished from living *D. rostriformis* (Fig. 2b–2j) by its larger size, reaching at least 33 mm (personal observation) as compared with 23 mm, more pronounced byssal groove, on average broader, more inflated shell, and often in having a color pattern of dark wavy lines on a light background. *Dreissena rostriformis* lacks a color pattern, except for slight darkening along the growth lines.

In addition to differing morphologically, *D. bugensis* and *D. rostriformis* differ in salinity tolerance. *Dreissena bugensis* typically occurs in freshwater, in salinities up to 1 ppt (Nevesskaya 1965); in the Dnieper/Bug Liman, it is killed by saline intrusions exceeding 6 ppt (T.G. Moroz, personal communication). As noted above, *D. rostriformis* does not occur in freshwater or in the northern parts of the Caspian Sea where salinity is lowered by flow of the Volga River; the species is restricted to the middle and southern Caspian, living in salinities up to 12.7 ppt (Zhadin 1952). Starobogatov (personal communication) now regards *D. bugensis* as a separate species because it cannot reproduce in water of the same salinity as Recent forms of *D. rostriformis* and consequently cannot interbreed. He states that *D. bugensis* reproduce in salinities below 2–3 ppt and *D. rostriformis* in salinities above 7–8 ppt. Although Starobogatov's recognition of *D. bugensis* as a full species is unpublished (see Table 2), it is sure to be influential among Russian taxonomists. Most recent Russian ecological and physiological studies of *D. bugensis* already treat it as a full species (e.g., Birger et al. 1978; Zolotareva et al. 1978; Pligin 1984; Alexenko and Alexandrova 1987; Lyashenko and Kharchenko 1987; Orlova 1987).

Babak (1983) reported *D. rostriformis bugensis* from Quaternary deposits of the Black Sea; previously it had been known only from the Recent fauna. She compared her Quaternary specimens with the type specimens of *D. bugensis* (Fig. 1a and 1b) and *D. tschaudae* var. *pontocaspica* Andrusov, 1897 (Fig. 2b–2g) at PIM and found them to be conspecific. Babak, however, included in synonymy with *D. r. bugensis* only those parts of the type series of *D. t. pontocaspica* from the Black Sea (Andrusov 1897, pl. 9, fig. 29–32; pl. 15, fig. 29 and 30), excluding specimens from the Caspian Sea (Andrusov 1897, pl. 9, fig. 27 and 28). The Caspian specimens were not located at PIM. Clarification of the status of *D. pontocaspica* must await designation of lectotype, which preferably should be one of the missing Caspian specimens. *Dreissena tschaudae* var. *pontocaspica* and *D. bugensis* were named on the same date, but if they are eventually proven synonymous, *D. bugensis* has priority, having been named as a full species rather than as a variety (International Code of Zoological Nomenclature, 1985, Article 57g).

Some fossil taxa that have been synonymized with *D. rostriformis* approach *D. bugensis* in morphology: *D. tschaudae* (Fig. 1g, lectotype) and *D. r.* vars. *gibba* (Fig. 1h, lectotype) and *vulgaris* (Fig 1i, lectotype). It may not be possible to draw a line between these taxa and *D. bugensis*,

FIG. 1. *Dreissena (Pontodreissena)* of the Black Sea Basin. Scale bar = 10 mm. (a) *D. bugensis*. Lectotype, PIM 451/77 (Andrusov 1897, pl. 15, fig. 33 and 37), Bug Liman near Nikolayev. 22.5 mm. (b) *D. bugensis*. Paralectotype, PIM 451/80 (Andrusov 1897, pl. 15, fig. 36.), Bug Liman near Nikolayev. 22 mm. (c) Zhadin's (1952) figure of *D. bugensis*; specimen is either an aberrant *D. bugensis* or *D. rostriformis* (compare Fig. 2e). (d and e) *D. bugensis*. Quagga mussels from Lake Erie. ANSP 398255. 34 and 19 mm. (f) *D. rostriformis rostriformis*. PIM 451/53. Andrusov's figured specimen (1897, pl. 14, fig. 3 and 4), which is quite similar to Deshayes' (1838) original (see Fig. 2a). Fossil, Kamyschburun. 33 mm. (g) *D. tschaudae*. Lectotype, PIM 451/75 (Andrusov 1897, pl. 15, fig. 24–25 and 28). Fossil, Mys Chauda, Crimea. 32 mm. (h) *D. rostriformis* var. *gibba*. Lectotype, PIM 451/57 and 451/194a (left and right valves of single individual) (Andrusov 1897, pl. 14, fig. 11–13 (13 is reversed)). Fossil, Kamyschburun. 36 mm. (i) *D. rostriformis* var. *vulgaris*. Lectotype, PIM 451/51 and 451/52 (left and right valves of single individual) (Andrusov 1897, pl. 14, fig. 5–7 (7 is reversed)). Fossil, Kamyschburun. 38.5 mm.

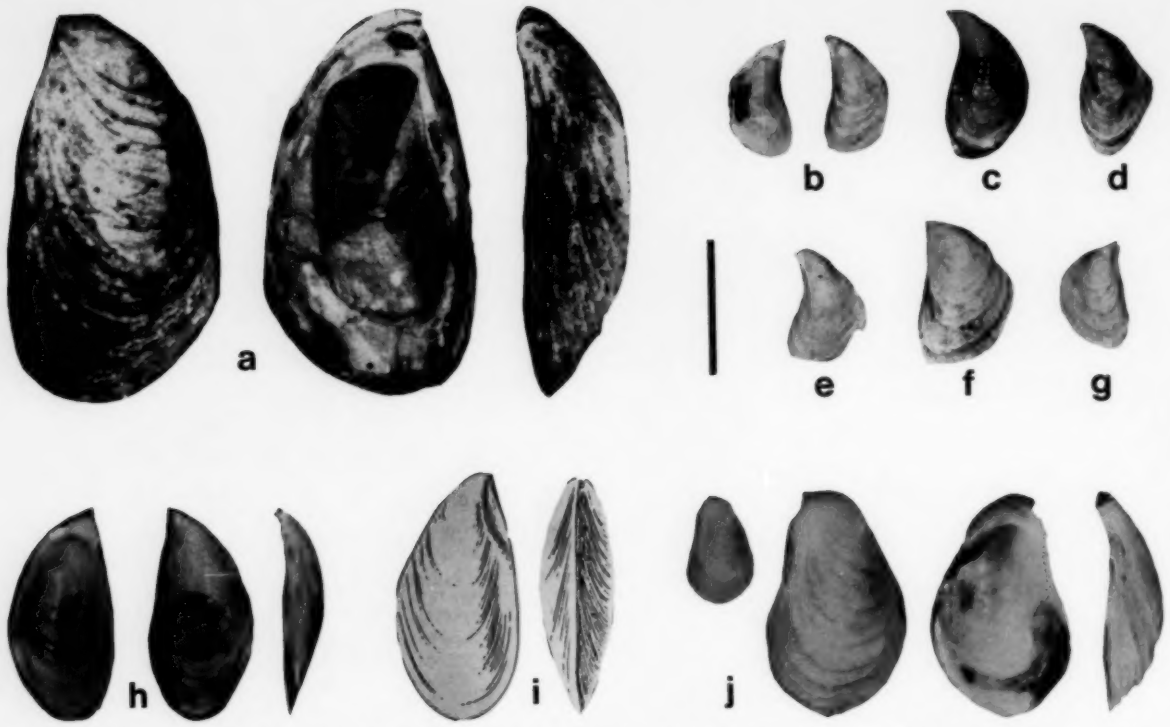

FIG. 2. *Dreissena rostriformis* varieties and subspecies. Scale bar = 10 mm. (a) *D. rostriformis rostriformis* Deshayes, 1838. Lectotype designated by, and figure reproduced from, Archambault-Guezou (1976, pl. 6, fig 2a–2c). Pliocene of Crimea. 28.5 mm. (b) *D. r.* var. *pontocaspica*. Syntype, PIM 451/40. Labelled at PIM as matching Andrusov (1897, pl. 9, fig. 28) but size (9 versus 17 mm in the figure) and shape are wrong. (c–g) *D. r.* var. *pontocaspica*. Syntypes, PIM 451/76, 451/44, 451/42, 451/43, 451/41 (Andrusov 1897, pl. 15, fig. 30; pl. 9, fig. 32, 30, 31, and 29), respectively. Subfossil, Black Sea. 9–13 mm. (h) *D. r.* var. *distincta*. Lectotype, PIM 451/60 (Andrusov 1897, pl. 14, fig. 24). Recent, Caspian Sea. 15.5 mm. (i) *D. r.* var. *compressa*. Reproduced from Logvinenko and Starobogatov (1966). Caspian Sea. 18 mm. (j) *D. r. grimmi*. Lectotype, PIM 451/81 (Andrusov 1897, pl. 16, fig. 16), leftmost to scale; other three twice scale. Caspian Sea. 8 mm.

although they are thicker shelled than the *D. bugensis* we have studied. There are two possible interpretations: (1) ancestral polymorphism in *D. rostriformis* has been sorted in the lineages descended from it, with the populations in the Caspian reaching smaller adult size and being on average more compressed, and the Dnieper/Bug populations, which invaded freshwater, reaching larger size and being on average more inflated, or (2) the distinction between *D. bugensis* and *D. rostriformis* was established by early Pleistocene, and *D. tschaudae* (from the Crimea) and *D. r.* vars. *gibba* and *vulgaris* (from Romania) are part of the *D. bugensis* lineage, which later became restricted to the Dnieper and Bug drainages. If this is the case, a question of priority of names arises, since all four names were introduced by Andrusov in 1897. *Dreissena rostriformis*, which was named as a full species, has priority over *gibba* and *vulgaris*, which were named as varieties of *D. rostriformis*. We here select *D. bugensis* to have priority over *D. tschaudae*, which was also named as a full species, in the event that they prove to be synonyms.

Why should *bugensis* among all the subspecies and varieties of *rostriformis* be raised to full species? At least in part because it has not been studied as thoroughly. (1) *Dreissena bugensis* until the 1940s was restricted to the Bug River and Liman and was little studied until it invaded the Dnieper River (Pligin 1984). (2) Logvinenko's (1965) extensive morphological studies included only species from the Caspian Sea and so did not consider *D. bugensis*. (3) Archambault-Guezou (1976), who did morphometric studies of *Dreissena* and synonymized many names under *D. rostriformis*, studied only fossil taxa and did not consider *D. bugensis*.

Marelli (1991) has recently suggested that *D. bugensis* and *D. polymorpha* are synonymous, but most recent authors have placed them in different subgenera, and their extensive fossil records clearly indicate that they are members of separate lineages (Taktakishvili 1973; L'vova and Starobogatov 1982; Babak 1983; Nuttall 1990a). Ludyanskiy (1991) summarized biochemical, ecological, and morphological data indicating that they are distinct species.

Dreissena polymorpha *(Pallas, 1771) (Fig. 3a–3d)*

Dreissena polymorpha ranges through most of Western Europe and the Baltic, Black Sea, Caspian, Northern Dvina River, and Arctic drainage basins (Zhadin 1952). Like *Dreissena rostriformis*, *D. polymorpha* is extremely variable in morphology and has been divided into several subspecies and varieties. Currently, several subspecies are recognized (Table 2), but the subspecies from the Aral Sea, *Dreissena polymorpha aralensis* Andrusov, 1897 (Fig. 3c) and

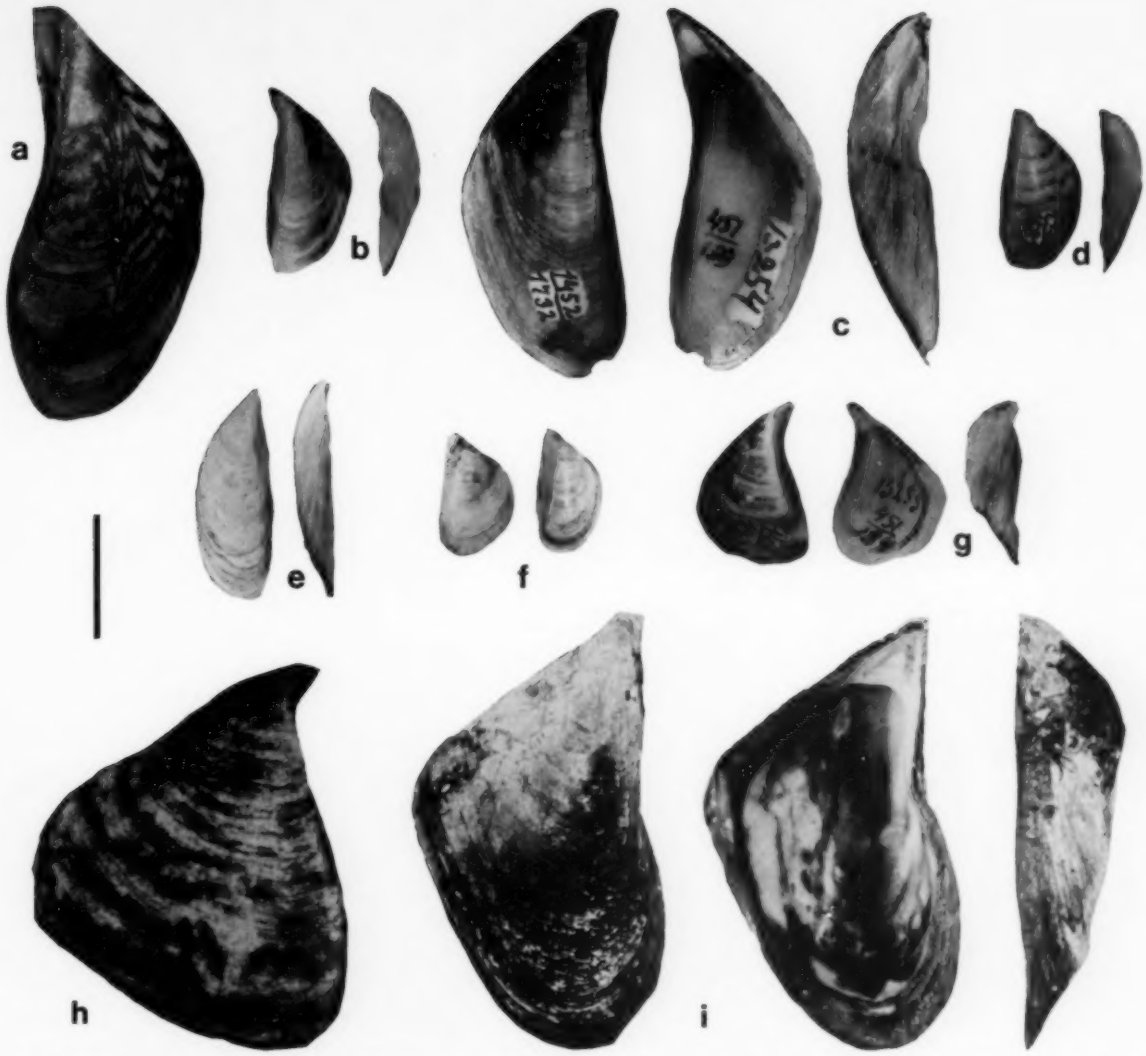

FIG. 3. Other *Dreissena* species. Scale bar = 10 mm. (a) *D. polymorpha polymorpha*. ANSP 55464. Lyons, France. 32 mm. (b) *D. p.* var. *andrusovi*. Lectotype, PIM 451/97 (Andrusov 1897, pl. 18, fig. 21 and 22). Caspian Sea. 15.5 mm. (c) *D. p. aralensis*. Syntype, PIM 451/159. Aral Sea. 29.5 mm. (d) *D. p. obtusecarinata*. Syntype, PIM 451/185. Aral Sea. 13 mm. (e) *D. caspia caspia*. PIM 451/32. Specimen figured by Andrusov (1897, pl. 7, fig. 21). Caspian Sea. 17.7 mm. (f) *D. caspia pallasi*. Syntypes, PIM 451/113, 451/115 (Andrusov 1897, pl. 20, fig. 33 and 35). Aral Sea. 10 and 10.5 mm. (g) *D. elata*. Syntype, PIM 451/184 (Andrusov 1897, pl. 20, fig. 25). Damysk, east coast of Caspian Sea. 13.5 mm. (h) *D. caputlacus*. Holotype, reproduced from Schütt (1993, fig. 14). Gölbaşi, Turkey. 30.8 mm. (i) *D. stankovici*. Lake Ohrid. Uncatalogued specimen, Natural History Museum, London. 36 mm.

D. p. obtusecarinata Andrusov, 1897 (Fig. 3d), are probably extinct because of the recent increase in salinity there caused by water diversion for irrigation; they were present in 1976, but not in 1980 (Aladin and Potts 1992). Possibly, *D. p. aralensis* survives in the mouth and delta of the Amu-Darja and Syr-Darja where those rivers enter the Aral Sea (Starobogatov, personal communication).

Recently, Ludyanskiy (1993) has suggested *Dreissena polymorpha andrusovi* Andrusov, 1897 (Fig. 3b) as a possible identity of the quagga mussel; we therefore concentrate on determining the status of this taxon in relation to *D. p. polymorpha*. Karpevitch (1955) found that *D. polymorpha* var. *marina* was a form morphologically transitional between *D. andrusovi* and *D. polymorpha* var. *fluviatilis*, as well as being intermediate in salinity tolerance. Logvinenko (1965) treated *D. andrusovi* as a subspecies of *D. polymorpha* because of the gradual transition from *D. polymorpha* in the Volga River to *D. andrusovi* in the northern Caspian Sea. Zhadin (1952, p. 53) cited the evolution of *D. andrusovi* "adapted to a specific salt content" from *D. polymorpha* as confirming the species concept of Lysenko. Karpevitch and Logvinenko in addition to Zhadin were Lysenkoists (see discussion under *D. rostriformis*), and we take their results to show ecophenotypic variation in a single variable species. Similarly, Shkorbatov (1986) investigated six populations of *Dreissena* along 1400 km of the Volga River drainage

(see map in Smirnova et al. 1993), recording oxygen consumption, salinity tolerance, and temperature tolerance along with 14 morphological measurements. He concluded that all populations represented a single taxon, *D. polymorpha polymorpha*, with populations being adapted to local conditions. We designate the specimen in Fig. 3b the lectotype of *D. p.* var. *andrusovi* to fix the application of the name.

The zebra mussel introduced into North America clearly corresponds to *Dreissena polymorpha*, not only morphologically, but also genetically (Boileau and Hebert 1993). Ludyanskiy (1993) noted that the RR "radially striped" phenotype illustrated by Smirnova et al. (1993), which is seen in some North American *D. polymorpha*, was known only in the Caspian and Aral seas. He suggested the Caspian as the source of *D. polymorpha* introduced into North America. In August 1993, we collected *D. polymorpha* in the Dnieper River and Dnieper/Bug Liman on the northern Black Sea and found that the radially striped phenotype occurs there also. The Black Sea is a more likely source than the Caspian for the introduction of *D. polymorpha* because North America does not receive ballast water directly from the Caspian (J. Carlton, personal communication).

Dreissena elata *Andrusov, 1897 (Fig. 3g)*

Andrusov named *Dreissena elata* as a variety of *D. polymorpha*. Logvinenko (1965) stated that *D. elata* did not occur in the northern Caspian Sea and that he had seen no forms transitional to *D. polymorpha*, and therefore recognized it as a good species. Logvinenko and Starobogatov (1968) reported *D. elata* as being extinct due to competition from invading *Mytilaster*. Starobogatov (personal communication) states that *D. elata* has not been collected since 1940–45 by more than a dozen expeditions in the Caspian.

Dreissena caspia *Eichwald, 1855 (Fig. 3e and 3f)*

Dreissena caspia is the only species of *Dreissena* other than *D. polymorpha* that has been regarded as a valid species since the time it was named. As with *D. elata*, *D. caspia caspia* (Fig. 3e) was reportedly driven extinct by invading *Mytilaster* and has not been collected since 1940–45. *Dreissena caspia pallasi* Andrusov, 1897 (Fig. 3f), is presumed extinct because of the recent increase in salinity of the Aral Sea caused by water diversion for irrigation; it was last recorded in 1980 (Aladin and Potts 1992).

Dreissena caputlacus *Schütt, 1993 (Fig. 3f)*

Dreissena caputlacus was named recently from a lake in southwestern Turkey (fig. 3, No. 20 in Kinzelbach 1992). The species has the broadest shell of living *Dreissena*. It may correspond to what Starobogatov (Table 2) calls *Dreissena diluvii* Abich, 1859 from the Euphrates and probably the Tigris drainages, but Schütt (1993) considered *D. diluvii* to be extinct. Schütt, however, did recognize *D. bourguignati* as a valid species whereas Starobogatov (personal communication) considers it a synonym of *D. diluvii*. Further research is needed on the status of *D. caputlacus*, *D. diluvii*, and *D. bourguignati* to determine if one, two, or three living species are represented by these names.

Dreissena stankovici *L'vova & Starobogatov, 1982 (Fig. 3g)*

Dreissena stankovici is apparently endemic to Lake Ohrid, which is typically freshwater in chemistry (Stanković 1960). The species has not been reported since it was named; however, several previously unidentified specimens of the species collected in 1935 are present in the collection of the Natural History Museum, London; one is illustrated here.

Other species

Kinzelbach (1992) has suggested that a number of relict species of *Dreissena* may survive in Greece and Asia Minor south of the Black Sea, and he recognized several species groups: *D. blanci* Westerlund, 1890, *D. bourguignati* Locard, 1883, *D. gallandi* Locard, 1893, and *D. anatolica* Locard, 1893. Schütt (1993) treated the first two of these as valid species and latter two as subspecies of *D. polymorpha*. Starobogatov (personal communication) also notes that the species status of *Dreissena* populations in Greece and Macedonia (e.g., *D. hellenica* Locard, 1893) needs investigation. Additional work is necessary to establish the status of these populations, however, on the basis of shell morphology, all of them belong to *Dreissena (Dreissena)* or *D. (Carinodreissena)*.

Identity of the Quagga Mussel

The following evidence points conclusively to *Dreissena bugensis* as being identical to the quagga mussel.

(1) Shells of some specimens of the quagga mussel in North America match type specimens of *Dreissena bugensis* Andrusov, 1897 in size, shape, and coloration; compare Fig. 1a and 1b with Fig. 1e.

(2) Species of *Dreissena (Dreissena)* and *D. (Carinodreissena)* (Fig. 3) can be excluded as possible identities for the quagga mussel, as they have a diagonal keel running the length of the shell. Some *D. caspia caspia* do not have a pronounced keel, but they are much slenderer than the quagga mussel.

(3) *Dreissena bugensis* is the only *Pontodreissena* reported in freshwater systems such as those that the quagga mussel is invading in North America. The only other living *Pontodreissena* is *Dreissena rostriformis* which occurs in the middle and southern Caspian Sea in salinities up to 12.7 ppt (Zhadin 1952). Living *D. rostriformis* are smaller than the quagga mussel, generally more compressed, and lack a color pattern.

(4) Allozyme profiles for *Dreissena bugensis* from the Dnieper River match those of the quagga mussel in North America (Spidle et al. 1994); preliminary allozyme work on *D. bugensis* that we collected last year confirms this conclusion (G. Rosenberg, in preparation).

Dreissena bugensis and *D. rostriformis grimmi* represent living lineages derived from *D. rostriformis rostriformis*. These lineages differ in morphology, coloration, maximum adult size, and salinity tolerance and are therefore regarded as separate species. It is possible that ancestral polymorphism in *D. rostriformis* has been sorted in the lineages descended from it, with the populations in the Caspian reaching smaller adult size and being on average more compressed and the Dnieper/Bug populations, which invaded freshwater, reaching larger size and being on average more inflated. Additional genetic, anatomical, and ecological research is needed to confirm the taxonomic status and relationships of the living species of *Dreissena*. If ranges of morphological variation in living species turn out to be concordant with species boundaries determined by allozyme electrophoresis or DNA sequencing, it might be possible to more reliably determine the status of names introduced for fossils. Pending completion of such revisionary work, we

recommend adoption of the name *Dreissena (Pontodreissena) bugensis* for the quagga mussel. This is the oldest name available for the *Pontodreissena* lineage in the Dnieper/Bug Drainage and it corresponds to the usage in the recent Russian ecological and physiological literature.

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Dr. Y.I. Starobogatov of the Zoological Institute in St. Petersburg kindly outlined his current views on *Dreissena* taxonomy in a letter dated 29 June 1993 and showed one of us (G.R.) his manuscript in press on dreissenid classification and the dreissenid collections at his institution. L.A. Nevesskaya allowed us to study Andrusov's collections at PIM. We benefitted from lively discussions of dreissenid taxonomy with a number of researchers in addition to Starobogatov and Nevesskaya: E.V. Babak and L.B. Ilyina in Moscow; Y.Y. Pligin and L.V. Shevtsova in Kiev, and T.L. Alexenko and T.G. Moroz in Herson. Moroz and V.S. Polishchuk arranged for us to do grab sampling in the Dnieper/Bug Liman. The Schileyko family graciously hosted us in Moscow. We thank Ellen Marsden, James Carlton, Adrian Spidle, and an anonymous reviewer for helpful comments on an earlier draft of this paper. This research was supported by the National Oceanic and Atmospheric Administration, grant NA26RG0403-01, Sea Grant Program.

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Identification of the Great Lakes Quagga Mussel as *Dreissena bugensis* from the Dnieper River, Ukraine, on the Basis of Allozyme Variation

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The discovery of a second dreissenid species, the quagga mussel, in the Great Lakes in 1991 prompted a search for its identity. We have identified the North American quagga mussel as *Dreissena bugensis* Andrusov on the basis of allozyme data and morphological characters. Further, a phenotypically distinct form of the quagga mussel found in Lakes Erie and Ontario also matches the electrophoretic profiles of the typical Lake Ontario quagga and European *D. bugensis*. We confirm that the white "profunda" mussel found in the deep waters of Lake Erie is a phenotype of the quagga mussel, and we conclude that the quagga mussel is *D. bugensis* which has been introduced from the Black Sea drainage of Ukraine.

La découverte en 1991 dans les Grands Lacs d'une deuxième espèce de dreissena, la moule quagga, a déclenché des recherches visant à préciser son identité. Sur la base de ses caractères morphologiques et des données relatives aux allozymes, nous avons identifié la moule quagga d'Amérique du Nord à l'espèce Dreissena bugensis Andrusov. Par ailleurs, une forme distincte sur le plan phénotypique de la moule quagga, que l'on retrouve dans les lacs Érié et Ontario, présente les mêmes profils électrophorétiques que celui de la moule quagga caractéristique du Lac Ontario et de la moule D. bugensis. Nous confirmons que la moule blanche des eaux profondes du Lac Érié est un phénotype de la moule quagga et nous concluons que la moule quagga appartient à l'espèce D. bugensis, qui a été introduite par le biais des eaux de la mer Noire, en Ukraine.

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The Laurentian Great Lakes have been subject to invasion by an increasing number of exotic species (Mills et al. 1993b). Few of these exotic species have led to immediately detectable effects on the ecosystem of the Great Lakes basin. A notable exception is the zebra mussel, *Dreissena polymorpha* (Pallas, 1771). It has affected food web interactions in the lakes (Hebert et al. 1991; Griffiths 1993) and also impacted industry by fouling water intake pipes along the lake shores. The quagga mussel, a second dreissenid species recently discovered Great Lakes (May and Marsden 1992), is likely to have additional, as yet unknown effects on North American ecosystems.

It has been difficult to acquire insight into the potential impact of the quagga mussel in North America because its identity is not yet known (May and Marsden 1992). Taxonomic identification of an invading species is critical for predicting the implications of its arrival in a new habitat. The lack of information about the characteristics of the quagga in its home range leaves no option but to determine its traits empirically. Preliminary studies on the quagga in the Great Lakes have indicated that it is clearly a species distinct from *D. polymorpha* (May and Marsden 1992) and that they do not interbreed in nature (Spidle et al., unpublished data). The quagga ranges from central Lake Erie to Quebec City on the St. Lawrence River and tends to inhabit deeper areas than the zebra mussel (Mills et al. 1993a). These data plus unpublished studies suggest that quagga and zebra mussels differ in their tolerance of temperature and salinity. The presence of the quagga is also associated with a possibly drastic change in the benthic community of Lake Erie (Dermott and Munawar 1993).

Sympatrically collected quagga and zebra mussels are morphologically distinct, as shown by comparison of samples collected in the upper St. Lawrence River (Fig. 1). Both species have a pointed anterior and a flared posterior end. The zebra mussel has a carina, a sharp angle between the side of the valve and a flat ventral surface, while the quagga is acarinate, with the valve curving into a rounded ventral surface. The identification of the quagga is complicated by the observation of a distinct *Dreissena* phenotype found in certain deep sections of Lakes Erie and Ontario, which has been termed "profunda" (Dermott and Munawar 1993). While Lake Ontario and St. Lawrence quagga mussels closely resemble *D. bugensis* from the Dnieper River,

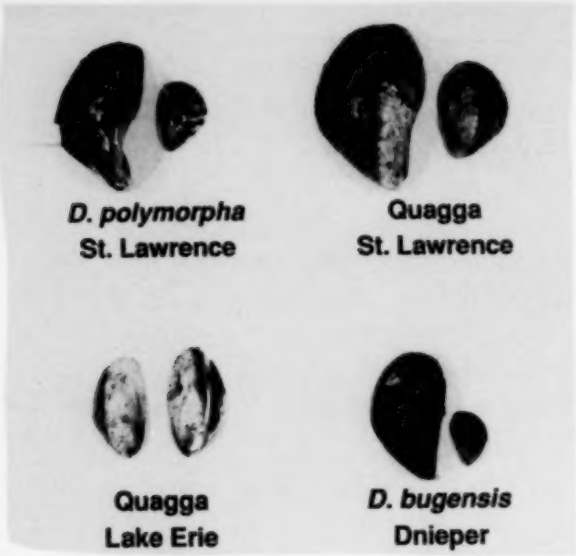

FIG. 1. Valves from samples of zebra and quagga mussels from the upper St. Lawrence River, the "profunda" phenotype of quagga from Lake Erie, and *D. bugensis* from the Dnieper River, Ukraine.

Ukraine, neither of these forms is very similar to the phenotype found in profundal zones of Lake Erie, which is most readily distinguished by its lack of a flaring posterior and its predominantly white coloration (Fig. 1). This paper reports the results of a study designed (1) to clarify the relationship between the quagga mussel and *D. bugensis* and (2) to determine if the "profunda" phenotype is indeed a third distinct species of *Dreissena* in the Great Lakes.

Methods

Sample Collection

A previously identified quagga mussel population was sampled by bottom trawling in the harbor of Cape Vincent, N.Y. (at the head of the St. Lawrence River). The "profunda" phenotype was collected by bottom trawling in southeastern Lake Erie. Two samples were obtained from the Dnieper River. The first sample, labeled "*D. polymorpha*", was collected in the Kakhovka Reservoir on the Dnieper River. They were received dissected, with their shells discarded. The second sample was composed of *D. bugensis* collected from Kherson near the mouth of the Dnieper River. These latter samples were preserved in ethanol and therefore were unavailable for protein electrophoresis. All other samples were packed on dry ice and shipped overnight to the Genome Variation Analysis Facility, where they were stored at −70°C until processed for electrophoresis.

Electrophoresis

Extracts of whole-body tissue and one valve were prepared, and the other valve from each animal, the Kakhovka Reservoir population excepted, was archived. Horizontal starch gel electrophoresis, histochemical staining, and banding pattern interpretation were carried out according to May (1992). Gene nomenclature for protein coding loci follows Shaklee et al., (1990). Individual zebra mussels from a population with known allele frequencies (Marsden et al. unpublished data.) were placed immediately adjacent to individuals from each population of quagga mussel to act as a standard reference for the scoring process. Eleven loci were scored. Four of these were polymorphic in the quagga mussel, i.e., the most common allele had a frequency less than 0.95 (Hartl and Clark 1989): glucose-6-phosphate isomerase 5.3.1.9 (*GPI**), isocitrate dehydrogenase 1.1.1.42 (*IDH**), phosphogluconate dehydrogenase 1.1.1.44 (*PGDH**), and Triose-phosphate isomerase 5.3.1.1 (*TPI**). The seven monomorphic loci were diaphorase 1.6.2.2 (*DIA**), formaldehyde dehydrogenase 1.2.1.1 (*FDH**), glyceraldehyde-3-phosphate dehydrogenase 1.2.1.12 (*GAPDH**), malate dehydrogenase 1.1.1.37 (*MDH1,2**), phosphoglucomutase 5.4.2.2 (*PGM1**), and inorganic pyrophosphatase 3.6.1.1 (*PP**). May and Marsden (1992) also used esterase 3.1.1.1 but this protein could not be reliably scored in the Ukrainian samples, presumably due to protein degradation from the length of time these samples had been stored.

Analysis

Sample sizes of 10 mussels from each North American population and 22 from the Kakhovka Reservoir population were chosen to ensure a reliable estimate of the genetic distance between the groups of mussels (Gorman and Renzi 1979). The software package "Genes in populations", designed by B. May and C.C. Krueger and written in C by W. Eng, was used for data analysis. Nei's genetic distance (Nei 1972) was calculated and used to construct a dendrogram by the UPGMA method. Allele frequency data from one quagga and four zebra mussel populations were used to provide reference points to demonstrate relatedness between putative quagga populations from Ukraine and Lake Erie, the known quagga population from the St. Lawrence River, and previously examined populations of zebra and quagga mussels. The zebra mussel populations were taken from Lakes Ontario and Erie as well as two sites in The Netherlands: the River Meuse near Roermand and Ijsselmeer near Enkhuizerzand. The quagga mussel population was from Lake Ontario near Rochester, N.Y. (May and Marsden 1992).

Results

Analysis of data from 11 allozyme loci indicates that the quagga mussel has the same genetic characteristics as *D. bugensis* and that they both match the Lake Erie deepwater phenotype. Allele frequencies and heterozygosity are both highly similar within, but not between, the quagga and zebra mussel populations (Table 1). The quagga mussel has one third the mean heterozygosity of the zebra mussel. The North American quagga mussel and the European *D. bugensis* match at those loci that are diagnostic between zebra and quagga mussels (*PP**, *PGM1**, and *MDH1**). At the two loci that are the most variable in each species the common allele for the quagga is essentially absent in the zebra mussel (*GPI*83* and *TPI*88*). For seven loci that are polymorphic in the zebra mussel, the common allele is absent in the quagga (*DIA*100, FDH*100, MDH1*100, MDH2*100, PGDH*100, PGM1*100,* and *TPI*100*).

The UPGMA dendrogram of Nei's genetic distances (Fig. 2) shows that the two putative and two known quagga mussel populations cluster together at a very short genetic dis-

TABLE 1. Allele frequencies at 11 allozyme loci in four quagga and four zebra mussel populations.

		Quagga mussel				Zebra mussel			
Locus	Allele	Kakhovka Reservoir	St. Lawrence	Lake Erie	Lake Ontario	Lake Erie	Lake Ontario	Ijsselmeer	River Meuse
N		22	10	10	21	243	158	40	40
*MDH2**	100	0.000	0.000	0.000	0.000	0.864	0.894	0.897	0.936
	140	1.000	1.000	1.000	1.000	0.136	0.106	0.103	0.064
*MDH1**	77	0.000	0.000	0.000	0.000	0.002	0.010	0.000	0.000
	100	0.000	0.000	0.000	0.000	0.591	0.587	0.679	0.808
	120	0.000	0.000	0.000	0.000	0.407	0.403	0.321	0.192
	122	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000
*PGM1**	88	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000
	96	0.000	0.000	0.000	0.000	0.002	0.004	0.000	0.000
	100	0.000	0.000	0.000	0.000	0.893	0.905	0.949	0.947
	107	1.000	1.000	0.950	1.000	0.002	0.004	0.000	0.000
	111	0.000	0.000	0.050	0.000	0.101	0.088	0.051	0.053
*IDH**	91	0.091	0.000	0.100	0.100	0.306	0.293	0.692	0.667
	100	0.909	1.000	0.900	0.900	0.694	0.703	0.308	0.333
	114	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.000
*TPI**	52	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	69	0.136	0.150	0.250	0.176	0.027	0.125	0.000	0.000
	86	0.023	0.050	0.050	0.088	0.000	0.000	0.000	0.000
	88	0.750	0.750	0.700	0.735	0.000	0.000	0.000	0.000
	100	0.000	0.000	0.000	0.000	0.683	0.566	0.513	0.551
	112	0.000	0.050	0.000	0.000	0.012	0.003	0.013	0.000
	115	0.000	0.000	0.000	0.000	0.259	0.293	0.474	0.449
	121	0.000	0.000	0.000	0.000	0.019	0.013	0.000	0.000
*DIA**	0	0.000	0.000	0.000	0.000	0.013	0.014	0.000	0.000
	100	0.000	0.000	0.000	0.000	0.623	0.683	0.973	0.969
	150	0.000	0.000	0.000	0.000	0.164	0.151	0.000	0.000
	160	0.000	0.100	0.000	0.000	0.030	0.018	0.000	0.000
	190	0.000	0.000	0.000	0.000	0.039	0.004	0.000	0.000
	200	1.000	0.900	1.000	1.000	0.131	0.130	0.027	0.031
*GPI**	−21	0.000	0.000	0.000	0.000	0.002	0.013	0.000	0.000
	50	0.000	0.000	0.000	0.024	0.017	0.013	0.013	0.000
	83	0.405	0.450	0.600	0.619	0.006	0.000	0.000	0.000
	100	0.000	0.000	0.000	0.000	0.297	0.331	0.329	0.395
	121	0.071	0.100	0.100	0.000	0.008	0.000	0.000	0.000
	146	0.190	0.050	0.000	0.000	0.488	0.433	0.447	0.421
	188	0.024	0.050	0.000	0.000	0.004	0.006	0.000	0.013
	196	0.310	0.350	0.300	0.357	0.176	0.191	0.211	0.171
	208	0.000	0.000	0.000	0.000	0.002	0.013	0.000	0.000
*PGDH**	100	0.000	0.000	0.000	0.000	0.924	0.877	0.671	0.553
	108	0.000	0.000	0.000	0.033	0.000	0.073	0.132	0.000
	123	0.958	0.950	0.900	0.967	0.074	0.050	0.197	0.447
	130	0.000	0.000	0.050	0.000	0.002	0.000	0.000	0.000
	165	0.042	0.050	0.050	0.000	0.000	0.000	0.000	0.000
*GAPDH**	97	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000
	100	1.000	1.000	1.000	1.000	0.701	0.578	0.359	0.244
	123	0.000	0.000	0.000	0.000	0.297	0.422	0.641	0.756
*FDH**	94	1.000	1.000	1.000	0.975	0.141	0.110	0.114	0.176
	100	0.000	0.000	0.000	0.025	0.859	0.890	0.886	0.824
*PP**	80	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000
	100	0.000	0.000	0.000	0.000	1.000	1.000	0.988	1.000
	110	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000
Average H_s		0.123	0.122	0.131	0.109	0.347	0.356	0.321	0.302
SE		0.069	0.066	0.058	0.054	0.060	0.063	0.065	0.063
Average H_0		0.101	0.127	0.145	0.106	0.341	0.325	0.291	0.291
SE		0.068	0.081	0.065	0.059	0.058	0.061	0.062	0.065

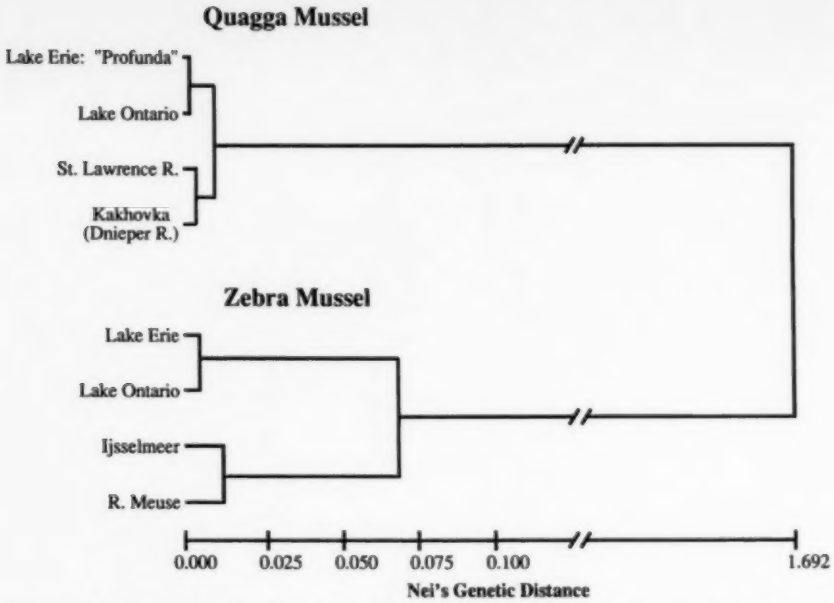

FIG. 2: UPGMA dendrogram of Nei's genetic distances between North American and European populations of zebra and quagga mussels based on 11 loci.

tance. The maximum genetic distance between quagga mussel populations is 0.005, where the cluster joining the "profunda" phenotype with the Rochester population meets the cluster joining the Ukrainian population with the upper St. Lawrence River population. The clusters of zebra mussel from North America and those from Europe are formed at genetic distances of less than 0.02, which is consistent with May and Marsden's (1992) results from the Great Lakes basin. The two continental clusters of zebra mussel join at a distance of 0.068, which is more distant, but well within the range to be expected from conspecifics with the amount of genotypic and geographic variability found in the zebra mussel (Avise and Smith 1977; Davis 1983; Thorpe 1983). In comparison, the zebra and quagga mussel populations cluster at a distance of 1.69.

Discussion

The quagga mussel is a member of the family Dreissenidae, possessing the diagnostic septum for the attachment of anterior adductor and anterior byssal retractor muscles (Morton 1993). The genus *Dreissena* is indicated by the lack of an apophysis above the septum. The systematics of this genus is in disarray. The Russian systematist Andrusov described eight living species of *Dreissena* in 1897. Western systematists have recognized one or two extant species: *D. rostriformis* (Andrusov, 1897) and/or *D. polymorpha* (Nuttall 1990; Marelli 1991). According to Rosenberg and Ludyanskiy (1994), four living species of *Dreissena*, with a varying number of subspecies, are currently recognized in the Russian systematic literature: *D. polymorpha*, *D. rostriformis*, *D. caspia* (Andrusov, 1897), and *D. elata* (described as a subspecies of *D. polymorpha* by Andrusov in 1897 and elevated to species level by Logvinenko in 1965). While *D. bugensis*, described as a species by Andrusov (1897), has been placed into a subspecies of *D. rostriformis* by current systematists, most Russian biologists retain Andrusov's classification (see Zolotareva et al. 1978; Pligin 1984). To conform to the body of research literature, we will use the name *D. bugensis* until an acceptable classification is worked out (see also Rosenberg and Ludyanskiy 1994).

Given the known range of *D. bugensis* and the fact that *D. polymorpha* and *D. bugensis* are the only members of the genus yet catalogued in the Dnieper River (Zhadin 1952), we conclude that the quagga mussel is synonymous with *D. bugensis*. Two pieces of evidence, one electrophoretic and one morphological, identify the quagga mussel as *D. bugensis*. Samples from the Kakhovka Reservoir showed allozyme patterns that matched loci diagnostic of the North American quagga rather than *D. polymorpha*. Morphological comparison was impossible because these samples were received without shells. The *Dreissena* population at this site was estimated to be 80–90% *D. bugensis* in 1977 (Pligin 1984). The second set of samples from the Dnieper River, identified as *D. bugensis* (T.G. Moroz, 32507 Kherson, Rabothaya, 76 a-144, Ukraine, personal communication), closely resembled the quagga mussel (Fig. 1).

The four populations of quagga mussel are closely matched at allozyme loci, confirming that the "profunda" type is a phenotypic variant and not another species. The profundal phenotype is genetically closer to the Ontario quagga mussel than to the Ukraine population (Fig. 2). Dermott and Munawar (1993) suggested that these quaggas from the deeper sections of Lake Erie resemble *D. distincta*, described by Andrusov in 1897. Our results, showing that the two phenotypes are conspecific, call Andrusov's description of *D. bugensis* and *D. distincta* as separate species into question.

The difference in the genetic distances between the new and old world populations of the two species of *Dreissena* is explained by the fact that *D. bugensis* has a much more limited range in Europe than *D. polymorpha* (Zhadin 1952).

Given that *D. bugensis* is only described in two rivers in the Black Sea drainage, the old world population would not be expected to show population substructure. Mills et al. (1993a) estimated that the quagga mussel arrived in the new world in 1989. This time span does not allow for significant differentiation from the founder population in the absence of a bottleneck. Since the *D. bugensis* population is not likely to be subdivided in the old world, we would expect the new world population to be quite similar to the old world population in its electrophoretic profile. Conversely, while the new world zebra mussel has also not had sufficient time to diverge from its source population, the old world zebra mussel population has had the time and space required to develop substructure. The genetic distance between the new world population of *D. polymorpha* and any unrelated old world populations would be expected to reflect this occurrence. The extent of genetic distance between the Dutch and North American zebra mussel indicates that the North American populations could not have arisen from these Dutch populations.

In the UPGMA dendrogram (Fig. 2) the cluster of four quagga mussel populations joins with the cluster of zebra mussel at a genetic distance of 1.69, which is greater than the value of 1.22 found by May and Marsden (1992). This increase in the estimate of genetic distance is partly due to the inclusion of populations of zebra mussel from Europe in the analysis which were not used by May and Marsden and partly due to the use of a different number of loci and alleles per locus. Thorpe (1983) and others (Avise and Smith 1977; Davis 1983) suggested that congeneric species will have a range of genetic distances between 0.16 and 1.39. Our finding that *D. polymorpha* and *D. bugensis* exceed that range may support their taxonomic classification into separate subgenera: *Dreissena* and *Pontodreissena*, respectively (Babak 1983; Nuttall 1990).

Identification of the quagga mussel as *D. bugensis* should stimulate researchers to investigate previous work which has been done on this species in its native range, in order to more readily predict its potential impacts in North America. Genetic confirmation of the existence of at least two species, *D. polymorpha* and *D. bugensis*, with two distinct phenotypes of the latter increases the taxonomic resolution of the genus. We verify that there is more than one extant species. The "profunda" phenotype indicates that considerable plasticity exists within these species. Ecological comparisons of the two extreme phenotypes of *D. bugensis* in the Great Lakes may lead to interesting insights into its morphological, and possibly physiological, plasticity.

Acknowledgments

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Genetic Zoogeography of the *Mysis relicta* Species Group (Crustacea: Mysidacea) in Northern Europe and North America

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The zoogeography and systematics of the *Mysis relicta* species group were elucidated in an allozyme survey of populations across northern Europe and North America. The North American populations are here identified as an independent species (sp. IV), distinct from the three previously recognized European *M. relicta* group taxa (spp. I–III). The geographical pattern of gene frequency variation in North America supports a late-glacial colonization by separate eastern and western refugial stocks of sp. IV. In Europe, sp. III is known from a single subarctic lake, while both spp. I and II are widespread. They coexist in the Baltic Sea, but their lacustrine distributions are largely different. Species I accounts for most Fennoscandian populations and those in Poland and Germany whereas sp. II lives in Ireland, parts of southwestern Scandinavia, and Karelia. With the paleohydrographical reference, the distributions suggest that both species survived the last glaciation in proglacial lakes east of the Scandinavian Ice. Subsequent distributional differentiation was influenced by environmental variations; the dispersal of sp. II in southwestern Scandinavia was facilitated by a broader euryhalinity than that in sp. I and other stenohaline "glacial relict" crustaceans. The Irish populations may represent a distinct refugial stock within sp. II.

Les caractéristiques zoogéographiques et systématiques du groupe spécifique *Mysis relicta* ont été dégagées par une étude allozymique des populations du nord de l'Europe et d'Amérique du Nord. Les populations nord-américaines sont ici identifiées comme constituant une espèce à part entière (esp. IV), distincte des trois taxons européens précédemment reconnus du groupe *M. relicta* (esp. I–III). Le profil géographique de la variation des fréquences géniques en Amérique du Nord est en accord avec l'hypothèse d'une colonisation tardiglaciaire par des stocks refugiens oriental et occidental distincts de l'esp. IV. En Europe, l'esp. III n'a été détectée que dans un seul lac subarctique; les esp. I et II sont l'une et l'autre très répandues. Elles coexistent dans la mer Baltique, mais ont toutefois des répartitions lacustres très différentes. L'espèce I représente l'essentiel des populations fennoscandiennes ainsi que celles de Pologne et d'Allemagne, tandis que l'esp. II se retrouve en Irlande, dans certaines régions du sud-ouest de la Scandinavie et en Carélie. Avec la référence paléohydrographique, les distributions semblent indiquer que les deux espèces ont survécu à la dernière glaciation dans les lacs proglaciaires à l'est de la Glace Scandinavie. La différentiation distributionnelle subséquente a été influencée par les variations environnementales; la dispersion de l'esp. II dans le sud-ouest de la Scandinavie a été facilitée par une euryhalinité plus étendue que celle de l'esp. I et d'autres crustacées sténohalins « reliques glaciaires ». Les populations irlandaises pourraient représenter un stock réfugien distinct au sein de l'esp. II.

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Fresh- and brackish-water mysid crustaceans conventionally ascribed to *Mysis relicta* Lovén have a wide distribution through the previously glaciated northern regions of Eurasia and North America. *Mysis relicta* has generally been treated as a prime example of the "glacial relict" faunal element, whose evolutionary and zoogeographical history has been the subject of extensive discussion and speculation (e.g., Lovén 1862; Samter 1905; Jägerskiöld 1912; Högbom 1917; Ekman 1922; Thienemann 1925, 1950; Segerstråle 1957, 1962, 1982; Holmquist 1959, 1966; Ricker 1959; Müller 1964; Kuderskii 1971; Dadswell 1974; Bousfield 1989; Väinölä and Varvio 1989). Features underlying the original notion of a relict status for *M. relicta* included the exceptional lacustrine habitats of a species from a primarily marine genus, an apparent preference for cold environments, and a distribution restricted to areas once inundated by coastal waters or by proglacial lakes associated with retreating continental ice, particularly by the early stages of the Baltic Sea.

The early relict concept assumed that the lacustrine populations were direct descendants of marine ancestors, left

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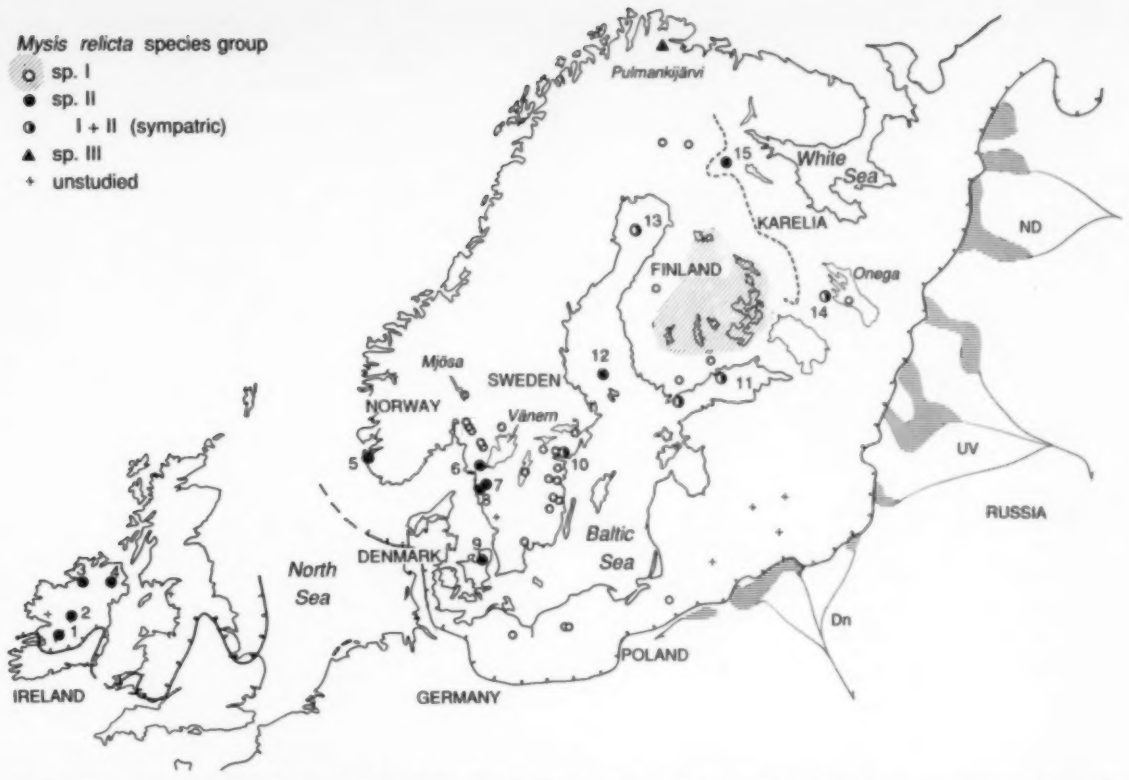

FIG. 1. Sampling sites in northern Europe, and the electrophoretically verified distributions of three *M. relicta* group species. The numbers refer to localities with sp. II, as indicated in Table 3. The oblique hatching represents records of sp. I from 86 lakes in the Finnish lake district; many of these sites were listed in Väinölä (1986) and Väinölä and Rockas (1990). The individual sp. I sites (open circles) in different countries are Unari, Pyhäjärvi of Pelkosenniemi, Lappajärvi, Lohjanjärvi, and Pyhäjärvi of Artjärvi in Finland; Mälaren, Yngaren, Båven, Hunn, Storsjön, Yxningen, Vättern, Hummeln, Stora Sinnern, Norra Bjärken, Björkern, Ivösjön, Mellan-Fryken, Ärtingen, and Lelången in Sweden; Mjøsa, Øgderen, Rødenessjøen, and Øymark in Norway; Breiter Luzin in Germany; Drawsko (=Dratzigsee), Żerdno, and Mamry (=Mauersee) in Poland; and Onega and Pertozero in Karelia. Some unstudied *M. relicta* group localities are shown (+) to illustrate the total northwestern European range, which also includes all larger lakes shown on the map. The maximum extent of the Late Weichselian glaciation (approximately 18 kya) is indicated with a hachured line. Location of major proglacial lake systems is shown schematically: ND, North Dvina; UV, Upper Volga; Dn, Dnepr headwaters (Kvasov 1979; Grosswald 1980). The broken line in eastern Fennoscandia shows the approximate position of the Baltic – White Sea watershed, impassable for *Mysis* in late- and postglacial times.

behind from Arctic seas that covered the present ranges at the end of the glacial period. *Mysis relicta* populations in different areas were thought to have been independently derived from a marine ancestor species (*M. oculata*) through parallel environmentally induced evolution (e.g., Lovén 1862; Samter 1905; Thienemann 1950). Dispersal to continental areas not flooded by coastal waters, such as much of the North American range and areas south and southwest of the Baltic Sea in Europe, was attributed to "sluicing up" in proglacial lakes dammed by (re)advancing ice sheets (e.g., Högbom 1917; Segerstråle 1957, 1976).

Today, most lacustrine *M. relicta* populations, and those in the brackish Baltic Sea, are regarded as descendants of freshwater refugial stocks that survived the glacial maximum along the southern margins of the major continental ice sheets (Segerstråle 1957, 1982; Ricker 1959; Dadswell 1974). However, there continue to be varying views on the origin, age, number, and relationships of the refugial stocks. Segerstråle (1957, 1982) has tried to reconstruct the immigration history of the "relicts" from their presumed site of origin in central Siberia through ice-dammed waters to northern continental Europe, to the Baltic Sea, and even to the British Isles, during the latest glaciation(s). He also still advocated a relatively recent, Late Pleistocene origin of the freshwater "relict" taxa. This view contrasts with that of Holmquist (1959, 1966), who regarded *M. relicta* as an ancient and coherent freshwater species with a history of lacustrine life dating back to the mid-Tertiary, and that of Bousfield (1989) on the ancient origins and systematic subdivisions of other "relict" crustacean taxa.

In our study, insight into the evolutionary and zoogeographical history of *M. relicta* is sought from patterns of genetic differentiation among populations and related species. In an earlier paper, the subdivision of the conventional *M. relicta* into three distinct sibling species in Fennoscandia was described (Väinölä 1986). The degree of allozyme differentiation suggested that the (monophyletic) *M. relicta* group probably diverged from its marine relatives some

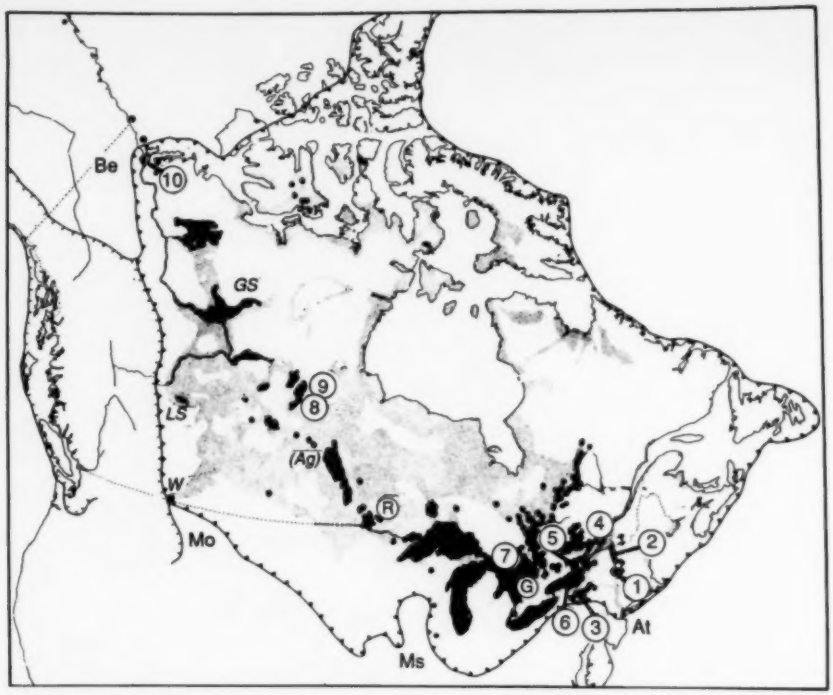

FIG. 2. Sample sites in North America, illustrating the electrophoretically verified distribution of sp. IV (circled numbers and letters). Names of the numbered lakes are given in Table 4. R = Roddy Lake, G = Gull Lake (identified from few individuals and an incomplete set of loci). The total distribution of the *M. relicta* group is shown in black (Holmquist 1966; Dadswell 1974). Shading indicates areas inundated by proglacial lakes and coastal waters during the deglaciation. The approximate maximum extent of the Late Wisconsinian glaciation and major freshwater faunal refuge areas are shown: At, Atlantic; Ms, Mississippian; Mo, Missourian; Be, Beringian (e.g., Bailey and Smith 1981). Other lakes indicated: W, Waterton Lake; LS, Lesser Slave Lake; GS, Great Slave Lake; (Ag), region once occupied by Glacial Lake Agassiz. Base map from Dadswell (1974), reproduced courtesy of the Canadian Museum of Nature, Ottawa, Ont.

million years ago in the Tertiary and that even the divergence of the sibling species (here referred to as spp. I, II, and III) possibly predated the Pleistocene.

The results supported the idea that the traditional "relict" element comprises subunits with different late- and post-glacial histories, in contrast with the view of a single late-glacial invasion of Europe from the east. The distributions of spp. I and II within the Baltic Sea and in lakes once isolated from it appeared to be differentially related to the present and past salinities, raising the hypothesis that their immigrations to this area were differentially related to the various fresh- and brackish-water phases during the evolution of the basin (Väinölä 1986). The discovery of distinct lineages also held promise for resolving the status and affinities of the disjunct *Mysis* populations in the British Isles and North America, which had been difficult to understand in the earlier discussions of "relict" origins.

We have now mapped the distributions of the sibling species throughout northwestern Europe and addressed their relationships to the North American freshwater populations, which prove to constitute a fourth genetically distinct species within the *M. relicta* group. We assess the late-glacial population history by relating distribution maps and patterns of intraspecific gene frequency variation on each continent to existing evidence on paleohydrographical connections of lakes and seas. The low dispersive capacities of the crustaceans concerned (inability to migrate upstream or to utilize external vectors) make them particularly suited to this approach: the current presence of a species in a lake provides evidence of its presence in that or an upstream area at the time of lake isolation, and the common genetic features in a set of populations of a similar age reflect the composition of the common ancestral stock in those times.

Material and Methods

Fresh material was collected throughout the range of the *M. relicta* group in northwestern Europe, mainly by a beam trawl from the deepest parts of lakes, and stored at −70°C prior to electrophoretic analysis (see Väinölä and Rockas 1990). The majority of samples are from central and southern Finland and Sweden, but many of the marginal European localities, in Ireland, southwestern Norway, Denmark, Germany, Poland, and northernmost Finland, are also included (Fig. 1). As regards lakes once isolated from the Baltic waters, attention was paid to sampling lakes of different ages. The isolation times of these lakes range from some 11.5 to 1.0 thousand years ago (kya). Three samples come from

Karelia (Russian Federation), including Lake Onega and one lake in the White Sea drainage area (Lake Paanajärvi). Material from a few sites in the northern Baltic Sea was also analyzed.

Samples were also electrophoretically studied from 12 North American localities. These comprise two of the Laurentian Great Lakes (Huron and Ontario) and four other sites in Ontario, three lakes in New York State, two in Manitoba, and one in the Mackenzie Delta area, Northwest Territories (Fig. 2).

The identification of European populations as sp. I, II, or III used the electrophoretic criteria (diagnostic enzymes) established in Väinölä (1986), here reproduced and supplemented in Table 1. To assess intraspecific gene frequency variation, some 120 specimens per locality were usually studied for the polymorphic enzymes (variation within sp. I is not treated in this paper, however). A set of 21 allozyme loci was used to confirm the identity of the European species in previously unstudied areas, to characterize the North American populations, and to compare the identity of alleles in the *M. relicta* group with those in the outgroup (see below). These loci were *Aco-2* (encoding aconitase), *Ark-1*, *Ark-2* (arginine kinase), *Dia-1*, *Dia-2* (diaphorase), *Eno* (enolase), *Gapd* (glyceraldehyde-3-phosphate dehydrogenase), *Got-1*, *Got-2* (glutamate oxaloacetate transaminase), *Gpi* (glucosephosphate isomerase), *Gpt* (glutamate pyruvate transaminase), *Idh-1*, *Idh-2* (NADP isocitrate dehydrogenase), *Mdh-2* (malate dehydrogenase), *Mpi* (mannosephosphate isomerase), *Pep-2* (peptidase, using the dipeptide leucyl-tyrosine), *Pgd* (6-phosphogluconate dehydrogenase), *Pgm* (phosphoglucomutase), *Pgk* (phosphoglycerate kinase), *Sdh* (sorbitol dehydrogenase), and *Tpi* (triosephosphate isomerase). The set of loci is slightly different from that in Väinölä (1986): additional loci involve *Gpt* and *Eno* whereas *Pep-1* and *Me-1* are not included. The electrophoretic methods used are described in Väinölä (1992).

The degree of genetic differentiation among species was characterized with Nei's measures of genetic identity and standard genetic distance (Nei 1987), and the neighbor-joining (NJ) algorithm was used to illustrate the estimated affinities in a phenogram (Saitou and Nei 1987). Relationships were also assessed through a direct examination of character state (allozyme) data in a phylogenetic analysis (e.g., Watrous and Wheeler 1981) using as an outgroup the three marine species of subgenus *Mysis* (*M. oculata*, *M. litoralis*, and *M. gaspensis*; data in Väinölä 1992). Alleles also recorded in the outgroup (with a frequency $p > 0.05$) were designated as plesiomorphic (ancestral) and alternative alleles found only within the *M. relicta* group as putative apomorphies (derived alleles); synapomorphy (uniquely shared apomorphy) provides evidence for monophyly. Inferred losses of putative plesiomorphs within the ingroup were given no phylogenetic significance; on the other hand, losses of putative apomorphs could be interpreted as homoplasy in this treatment.

Intraspecific variation was quantified using the standardized allele frequency variance measure F_{ST}, estimated with Weir and Cockerham's (1984) formula for panmictic local populations; this was also used as a pairwise interpopulation distance measure. For moderate distances ($F_{ST} < 0.2$), differentiation due to genetic drift is expected to be approximately proportional to accumulated inverse effective population size: $F_{ST} \simeq {}^1/_2 \Sigma_t N_e^{-1}$. In the longer run, for isolated populations of constant size, the expectation is $F_{ST} = 1 - \exp(-t/2N_e)$ (e.g., Reynolds et al. 1983). The relative affinities of populations were visualized by ordination through linear multidimensional scaling of the F_{ST} distance matrix, using the SPSS[x] program package.

TABLE 1. Prevalent alleles (allozymes) at differentiated loci in the four *M. relicta* group species.[a] Alleles found only at frequencies <0.25 are in parentheses. Putative apomorphies are in bold. Other alleles are generally plesiomorphic (i.e., also present in the outgroup comprising marine species of the subgenus *Mysis*); the outgroup species sharing a given plesiomorphic allele are indicated in the last column (*g* = *M. gaspensis*, *l* = *M. litoralis*, *o* = *M. oculata*). However, the polarity of allele states at *Ark-1* and *Gpi* cannot be assigned with the outgroup information. Data on allele frequencies are given for spp. II and IV in Tables 3 and 4 and for spp. I and III in Väinölä (1986, 1992) (see also Fig. 5b).

Locus	Species I	Species II	Species III	Species IV	Outgroup species
Aco-2	***(104)***	***104***			
	100		*101*	*101*	*l*
Ark-1	*100*	*100*	*100*	*92*	
Dia-1	*100*	*100*	*100*	***99***	*l, o*
Got-1	***100***		*96*	***100***	*g, l*
		92	*92*		*o*
Got-2		*105*		*105*	*l, o*
	100			***100***	
			98		*g, l*
Gpi	*100*	*100*	*104*	*97*	
			93	*90*	
Gpt			*107*	*107*	*o*
	100	*100*		***95***	*g, l*
Idh-2	***(109)***				
	100	*100*	*100*	*100*	*g, l*
Mpi	*107*	*107*	*107*	*107*	*l, o*
	100	*102*			*g*
Pep-2	***100***			***100***	
		93	*93*		*g*
		91			
Pgd		***(103)***	***(103)***		
	100	*100*	*100*	*100*	*l, o*
Pgm			*105*		*o*
	104				*l*
	100	*100*		*100*	*g*
				92	
Tpi	*100*	***(102)***			*l*
	90	*90*	*90*	*90*	*g*
		80	*80*		*o*

[a]The following loci were undifferentiated in the four species: *Ark-2, Dia-2, Eno, Gapd, Idh-1, Mdh-2, Pgk, Sdh.*

Results

Diagnosis of Species

The observed genetic differences among species are summarized in Table 1, which shows the prevalent alleles (i.e., those with an average frequency in a species greater than

TABLE 2. Pairwise estimates of genetic identity *I* (below diagonal) and standard genetic distance *D* (above diagonal; SE in parentheses) for the four *M. relicta* group species, based on data from 21 loci. Estimates are based on average gene frequencies of populations in Tables 3 and 4 for spp. II and IV and the data in Väinölä (1986) for spp. I and III.

	Species			
Species	I	II	III	IV
I	—	0.27 (0.12)	0.47 (0.17)	0.40 (0.15)
II	0.76	—	0.32 (0.13)	0.51 (0.17)
III	0.63	0.73	—	0.37 (0.15)
IV	0.67	0.60	0.69	—

0.05) at loci exhibiting interspecific variation. The three European species (spp. I–III) are separated from one another by two to seven completely diagnostic loci (confidence of correct species assignment from single-locus genotype >99.9%) and by a few additional ones with species-specific alleles at high frequencies. The distinction of sp. III from the others at *Gpt* and the recognition of the variant allele *Pep-2*^{91} in sp. II extend the previous data on differentiating characters (Väinölä 1986).

The North American populations have a common characteristic set of alleles distinguishing them from all the European species. They are fixed (*Ark-1*, *Dia-1*) or polymorphic (*Gpi*) for unique alleles at three loci, and unique alleles are also found at high frequencies at *Gpt* and *Pgm* (cf. Fig. 5b). The number of diagnostic loci between the American populations and any of the European species (five to seven) is similar to that among the latter. In terms of genetic distances *D*, the mean divergence of the American populations from the three European species is 0.43 (range 0.37–0.51), while that among the European species is 0.36 (0.27–0.47) (Table 2). The *D* estimates among individual American populations, or those within any European species, do not exceed 0.06 (Fig. 5a). On these grounds, we conclude that the North American freshwater populations constitute another distinct species of the *M. relicta* species group; it will be referred to here as sp. IV.

Distributions

The electrophoretically verified distributions of the three European species are shown in Fig. 1. Species III is only known from a single locality, Lake Pulmankijärvi in northern Lapland, on the Finnish–Norwegian border (Väinölä 1986). The lake lies some 17 m above sea level (asl) and discharges to Tanafjorden in the Barents Sea. We checked three other lakes at similar altitudes and latitudes in northern Norway, but found no *Mysis*.

Species II accounts for the southwestern part of the range of the *M. relicta* group in northern Europe, but it is also present in some of the most easterly localities examined. It is the only species found in the four Irish lakes (localities 1–4 in Fig. 1), in the single known *Mysis* localities of southwestern Norway (5) and Denmark (9), in three lakes near the Swedish west coast (6, 7, 8), and in Lake Paanajärvi (15) in the White Sea drainage. In addition, it has been found sympatrically with sp. I in Lake Båven (10) near the eastern (Baltic Sea) coast of Sweden (Väinölä 1986) and in Lake Pertozero (14, draining to Lake Onega) in Karelia. The two species also coexist in the Baltic Sea; the earlier suggestion that sp. II is prevalent in the open sea and outer archipelago habitats in this basin (Väinölä 1986) is confirmed by the new data.

The distribution of sp. I comprises most of the central and eastern parts of the European range of the *M. relicta* group. It was identified in 91 lakes in Finland (i.e., all studied Finnish populations except Pulmankijärvi), in 15 central and southern Swedish lakes, and in four lakes of southeast Norway. It is also present in continental lakes south of the Baltic in Poland and Germany and in the Lake Onega region in East Karelia (Fig. 1). In the Baltic, sp. I is the predominant species in coastal and peripheral areas, but is also often found in sympatry with sp. II, particularly in the Bothnian Bay.

All 12 North American populations examined were attributable to sp. IV; the distribution of the study sites spans most of the North American continental range of freshwater *Mysis* (Fig. 2).

Differentiation within Species

Intraspecific gene frequency variation is here described in detail for the European sp. II (Table 3) and the North American sp. IV (Table 4). An account of differentiation within sp. I will be presented elsewhere. Gene frequencies for a representative sp. I site (from the Baltic) and for the single known sp. III population are given in Väinölä (1986).

Species II

On the basis of data from seven variable loci (Table 3), the sp. II material falls into three main groups (Fig. 3a): (i) the Irish populations (1–4), (ii) the southwestern Norwegian Lake Stokkelandsvatn population (5), and (iii) populations in Sweden, Denmark, the Baltic Sea, and Karelia (eastern group, 6–15).

The Irish and eastern groups are most clearly distinguished by *Pep-2*, with different alleles predominating in each area. *Pgd* and *Got-1* also contribute to this distinction. The two most polymorphic loci, *Mpi* and *Tpi*, show little differentiation between these two groups, but the Stokkelandsvatn population deviates from the others by being monomorphic, or nearly so, at both these loci. In terms of F_{ST}, the differences among the three main groups are approximately 0.30 (Fig. 3a).

As regards variation within the main groups, samples from single drainages (1 and 2 in Ireland, 7 and 8 in Sweden) or from different parts of the northern Baltic (11-13) show no significant differences (connected sites in Fig. 3a). Irish populations from different drainages are moderately differentiated at *Mpi* and *Tpi* (overall $F_{ST} = 0.03$).

TABLE 3. Allele frequencies (%) at polymorphic loci in samples of sp. II. + denotes a sample frequency <0.005. *N* is the number of individuals studied.

		Area, location, and sample code														
		Ireland				Southwestern Norway	Southwestern Sweden			Denmark	Eastern Sweden	Baltic Sea			Karelia	
Locus	Allele	Lough Derg 1	Lough Ree 2	Lough Erne 3	Lough Neagh 4	Stokkelandsvatn 5	Öresjön 6	Mjörn 7	Aspen 8	Furesø 9	Båven 10	Gulf of Finland 11	Bothnian Sea 12	Bothnian Bay 13	Pertozero 14	Paanajärvi 15
Got-1	*96*	—	—	—	—	—	7	8	11	+	7	3	2	5		18
	92	100	100	100	100	100	93	92	89	100	93	97	98	95		82
	(*N*)	(80)	(70)	(76)	(71)	(44)	(131)	(138)	(99)	(109)	(149)	(125)	(146)	(101)	(—)	(129)
Got-2	*105*	97	97	95	97	100	100	100	100	100	100	99	99	98	96	99
	100	3	3	5	3	—	—	—	+	+	+	1	1	2	4	1
	(*N*)	(137)	(132)	(120)	(130)	(136)	(140)	(128)	(167)	(126)	(149)	(164)	(146)	(81)	(12)	(132)
Mpi	*107*	32	33	45	58	95	37	21	27	57	21	32	35	33	9	21
	102	68	67	55	42	5	63	79	73	43	79	68	65	67	91	79
	(*N*)	(128)	(109)	(129)	(118)	(162)	(126)	(114)	(118)	(126)	(145)	(139)	(142)	(116)	(11)	(129)
Pep-2	*93*	30	34	28	20	52	100	99	97	99	89	85	84	90	100	100
	91	70	66	72	80	48	+	1	3	1	11	15	16	10	—	—
	(*N*)	(106)	(91)	(100)	(96)	(110)	(112)	(106)	(119)	(131)	(138)	(81)	(143)	(97)	(12)	(116)
Pgd	*103*	+	1	3	3	1	22	17	22	6	22	19	18	15		2
	100	100	99	97	97	99	78	83	78	94	78	81	82	85		90
	93	—	—	—	—	—	—	—	—	—	—	—	+	—		8
	(*N*)	(102)	(108)	(118)	(71)	(91)	(110)	(95)	(82)	(106)	(88)	(103)	(133)	(78)	(—)	(126)
Pgm	*104*	1	—	—	+	+	+	2	1	—	1	2	2	2	23	9
	100	99	99	97	99	100	98	97	92	100	98	97	97	98	69	86
	96	+	1	3	1	—	1	1	7	+	1	1	1	1	8	5
	(*N*)	(129)	(132)	(144)	(131)	(151)	(103)	(107)	(113)	(128)	(138)	(99	(146)	(98)	(13)	(121)
Tpi	*102*	9	8	18	8	—	12	12	13	4	9	13	8	8	—	—
	90	61	65	53	70	100	55	44	43	52	26	55	55	50	61	53
	80	29	27	29	22	—	33	42	41	43	65	29	35	38	39	47
	65	—	—	—	—	—	—	2	4	1	—	3	1	4	—	—
	(*N*)	(124)	(128)	(141)	(120)	(157)	(120)	(116)	(124)	(125)	(120)	(132)	(106)	(77)	(14)	(125)

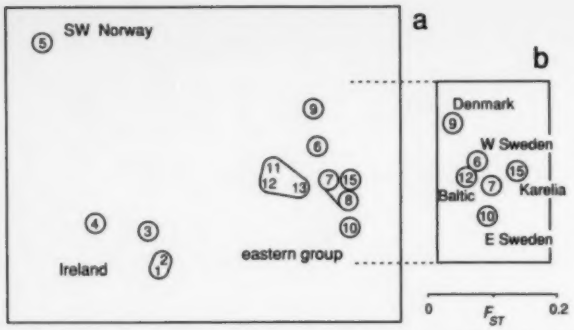

FIG. 3. (a) Ordination of sp. II samples by linear two-dimensional scaling on the basis of genetic distances. The squared correlation of the original (F_{ST}) and plot distances is $r^2 = 0.98$. Samples that showed no statistically significant ($p < 0.05$) allele frequency differences are interconnected. (b) As the interrelationships of closely related populations tend to be somewhat distorted and inflated in the ordination, another analysis was performed on the eastern population group only, after removing statistically indistinguishable samples; $r^2 = 0.99$. Approximate scale, common to the two plots, is from a regression of the original on plot distances.

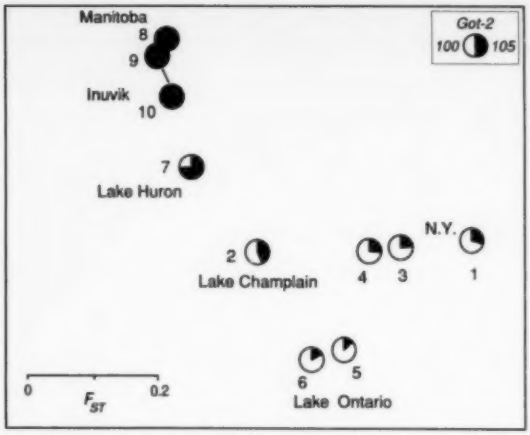

FIG. 4. Ordination of sp. IV samples by linear two-dimensional scaling on the basis of F_{ST} distances; $r^2 = 0.97$. Samples with no significant allele frequency differences are interconnected. Pie diagrams illustrate the allele frequencies at the *Got-2* locus.

Within the eastern group, the Baltic stock is most similar to the Swedish west coast populations ($F_{ST} = 0.02$ among the two drainages and the Baltic, sites 6, 7, and 12 in Fig. 3b). Each of the remaining populations in this group has some unique features (but sample 14 is too small for conclusive interpretations and not distinguishable from 15). The Danish sample (9) deviates from the general Baltic–Kattegat composition in *Mpi* and *Pgd* frequencies and lacks some rare alleles at other loci. The eastern Swedish population (10) differs most clearly at *Tpi*. The four populations in the Kattegat area (6–9) as a whole are distinguished from the Baltic and Båven populations (10–13) through virtual monomorphism at *Pep-2*. The Karelian sample(s) (14, 15) feature higher polymorphism or different variant alleles at *Pgd*, *Pgm*, and *Got-1*; however, they lack some of the variation found at *Tpi* and *Pep-2* in the Baltic–Kattegat area. Distances among these moderately diverged eastern subgroups range from a minimum F_{ST} of 0.04 to a maximum of 0.11 (see Fig. 3b).

Species IV

In North America, the main pattern of differentiation is seen on an east-to-west axis. This is most evident in *Got-2* allele frequencies (Table 4; Fig. 4): $Got\text{-}2^{105}$ is fixed in central and northwestern Canada (8–10), but occurs only at frequencies of 0.13–0.43 in the east (New York and Ontario). A parallel geographical pattern is found at *Pgm*. The transition from northwestern to eastern populations through Lake Huron (7) is gradual, with the Huron population showing a stronger affinity to the northwest. Among the eastern populations, that of Lake Champlain (2) appears to be genetically closer to the western group than do the others (Fig. 4).

Below this large-scale pattern (average east–west distance $F_{ST} = 0.37$), relatively large frequency differences were observed among the eastern sites (samples 1–6: $F_{ST} = 0.11$). The George (1) and Cayuga (3) populations are linked by high frequencies of the Pgm^{92} allele and are also closely allied with the Big Rideau Lake (4) population. Populations around Lake Ontario show irregular differences, e.g., the Lake Mazinaw population (5) is monomorphic at two loci (*Mpi*, *Gpi*) which are variable in most other populations.

Relationships among Species

In terms of overall differentiation, as reflected in the genetic distances (Table 2) or numbers of diagnostic loci, the least divergence is seen between spp. I and II (two completely diagnostic loci, six diagnostic at the 95% level; cf. Fig. 5a). The clearest distinctions (seven completely diagnostic loci) are between the American sp. IV and the European spp. II and III and between spp. I and III. However, the interspecific distance estimates are not significantly different from one another (Table 2), and little confidence can be placed on the branching pattern of the distance-based phenogram (Fig. 5a). For instance, bootstrapping over loci reconstructed spp. I and II as the most similar species pair in only 51% of replicate samples.

From an outgroup analysis of allozyme character polarity, the species pair (I, IV) gets the strongest support as a terminal clade. These species have putative synapomorphic alleles at three loci (*Got-1, Got-2, Pep-2*) (Table 1; Fig. 5b); at two of these loci the alleles are monomorphic in both species. There are single candidate synapomorphic alleles for species pairs (I, II) ($Aco\text{-}2^{104}$) and (II, III) (Pgd^{103}), but they are only found at a low frequency in one or both species.

In addition to the synapomorphies, spp. I and IV also have more autapomorphies than the other two species (Fig. 5b). Overall, sp. IV has apomorphic alleles at eight of the 13 differentiated loci, exclusively at 5. By contrast, sp. III has plesiomorphs at 12 loci, and only one putatively exclusively autapomorphic locus *Gpi* (with the hypothesis of Fig. 5b, the actual differences between spp. II and III are mostly interpreted as segregation of ancestral polymorphisms). Note that the inferred polarity of *Gpi* and *Ark* alleles in Fig. 5b is conditional on the particular tree topology: no alleles are shared with the outgroup at these loci (Table 1).

TABLE 4. Allele frequencies (%) at polymorphic loci in samples of sp. IV.

		Location and sample code									
		New York			Ontario				Manitoba		Northwest Territories
Locus	Allele	George 1	Champlain 2	Cayuga 3	Big Rideau 4	Mazinaw 5	Ontario 6	Huron 7	Southern Indian 8	North Knife 9	Inuvik 10
Got-2	*105*	29	43	23	26	13	16	74	100	100	100
	100	71	57	77	74	87	84	26	—	—	—
	(*N*)	(79)	(60)	(75)	(99)	(82)	(16)	(84)	(20)	(16)	(14)
Gpi	*104*	13	—	26	25	—	6	13	6	3	—
	90	87	100	74	75	100	94	88	94	97	100
	(*N*)	(91)	(88)	(107)	(111)	(107)	(17)	(96)	(33)	(17)	(14)
Gpt	*107*	22	30	17	10	34	14	12	11	6	7
	95	78	70	83	90	66	86	88	89	94	93
	(*N*)	(70)	(86)	(89)	(95)	(88)	(7)	(85)	(33)	(17)	(7)
Mpi	*111*	1	2	1	+	—	—	6	8	6	—
	107	99	98	98	99	100	97	94	89	94	100
	104	—	—	1	+	—	3	—	3	—	—
	(*N*)	(91)	(90)	(90)	(111)	(110)	(17)	(96)	(33)	(17)	(14)
Pgm	*100*	86	21	64	49	41	24	12	2	—	11
	92	14	71	35	49	59	76	87	98	100	89
	87	—	7	1	2	—	—	1	—	—	—
	(*N*)	(91)	(89)	(115)	(111)	(103)	(17)	(95)	(33)	(17)	(14)

Discussion

Relationships among Species

The distributions of the *M. relicta* group species have now been widely mapped in northwestern Europe and across continental North America. However, the northernmost parts of the range (brackish- and freshwater populations in Arctic Canadian islands, Alaska, Siberia, Spitzbergen, and East Greenland; Holmquist 1959, 1966, 1975; Dadswell 1974) remain unexplored. The closest affinities of the so far unique sp. III population in Lake Pulmankijärvi, isolated from the Barents Sea only some 5 kya (Møller 1987), are likely to be to the populations in these areas. In any case, the present distributional data give few clues to the identity of populations linking the European and North American distributions along the Siberian coast.

Rare hybridization (0.6% F1) between spp. I and II has been detected in their area of sympatry in the Bothnian Bay (R. Väinölä and J.K. Vainio, unpublished data). However, no evidence of effective local gene exchange between the lineages is seen in the current population structures, and the full specific distinction remains unequivocal. The other species were only found in allopatry; their species status is justified by the comparative evidence on amount of divergence, which is no smaller than that between spp. I and II and similar to that among species-level taxa in many other animal groups (e.g., Thorpe 1983).

Alternative methods for assessing the relative affinities of the four species gave somewhat discordant results: the outgroup analysis suggests spp. I and IV as a terminal clade whereas spp. I and II are the closest phenetically. Yet, even though the phylogeny could not be conclusively resolved, some alternatives seem particularly unlikely. No putative synapomorphies were found for three of the six possible species pairs, i.e., (I, III), (II, IV), and (III, IV). Also, considering pairwise allele sharing at the purely phenetic level (disregarding the outgroup), pairs (I, III) and (II, IV) have no uniquely shared alleles, while all other pairs have three or four.

Similar results on the relative affinities of species emerge from morphology. A canonical analysis of morphometric data (B.J. Riddoch and R. Väinölä, unpublished data) unequivocally separated the three species pairs which share no putative synapomorphic alleles whereas some overlap (<5%) remained for the pairs (I, II), (I, IV), and (II, III). Species IV thus appears closer to sp. I than to the other European species in both data sets.

The systematic and zoogeographic significance of the subdivision in the *M. relicta* group may also be examined in the light of results from other similarly distributed fresh- and brackish-water organisms. Data on Holarctic coregonid fishes and on another "relict" crustacean group, the pontoporeid amphipods, suggest that on broader temporal and spatial scales, the evolutionary and dispersal histories of these taxa may actually have little in common with *Mysis*.

The divergence among the *M. relicta* group species generally exceeds that among well-established *Coregonus* species (e.g., *C. lavaretus*, *C. peled*, *C. autumnalis*, *C. albula*: mean $D = 0.20$, range 0.10–0.30) and is more similar to that between coregonid genera (*Coregonus* and *Stenodus*: $D = 0.31$) (Bodaly et al. 1991; but cf. Ferguson et al. 1978). Indeed, the largest differences within individual *Mysis* species (between postulated refugial stocks) are similar to those between some conventionally recognized allopatric *Coregonus* species on different continents, e.g., the lake whitefishes *C. lavaretus* and *C. clupeaformis*. (Bodaly et al. (1991) actually questioned the species status of these and of some other vicarious coregonid pairs on genetic grounds.). In general, the distinction

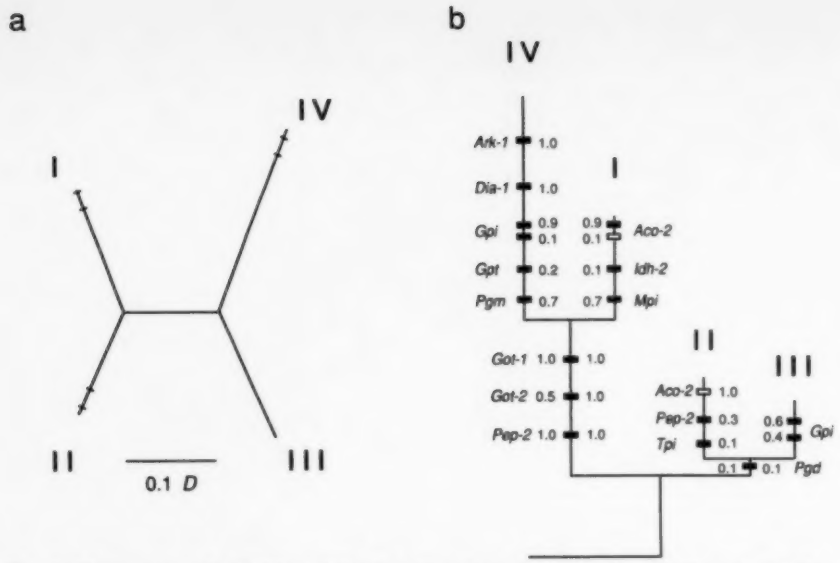

FIG. 5. Divergence and affinities of the four *M. relicta* group species. (a) Phenetic relationships of species illustrated by NJ clustering on the basis of genetic distances *D* (Table 2). The maximum and mean level of differentiation within each species are illustrated with cross-bars at half the respective distance from the tip of a branch. (b) A cladogram suggested by an outgroup analysis of allozyme relationships. Putative apomorphic alleles and their average frequencies in each species are shown (open boxes represent homoplasy; see Table 1 for allele names). Branch lengths reflect the total frequency of deduced apomorphic alleles.

between intra- and interspecific levels of differentiation in *Mysis* (Fig. 5a) is clearer than in *Coregonus*. Such comparisons reinforce the view that the origin and diversification of the *M. relicta* group did not occur in connection with the latest Pleistocene glaciations but earlier, probably several hundred thousand or even millions of years ago (Väinölä 1986). Along with the great divergence, the (probably) disjunct ranges of the European and North American *Mysis* spp. also suggest that the long-range and long-term dispersal in *Mysis*, over the last few glacial cycles, has been considerably more restricted than that of *Coregonus* spp., as even more closely related coregonid taxa may each have practically circumpolar distributions.

In contrast with the closer relationships in coregonids, allozyme divergence between Eurasian and North American freshwater pontoporeids ($D \simeq 1.5$; Väinölä and Varvio 1989) is much greater than that between the *M. relicta* group spp. ($D < 0.5$). A higher level of taxonomic separation for the old-world and new-world pontoporeids (i.e., genera *Monoporeia* and *Diporeia*; up to 1977, these were treated as conspecific) has also been suggested on morphological grounds (Bousfield 1989).

The common history of the various "glacial relict" taxa in the late-glacial time, reflected in their largely coincident present-day distributions, thus does not seem to extend to more remote zoogeographical events such as the marine-to-freshwater habitat shifts and to later lineage splitting leading to emergence of the sibling species. The North American *Diporeia hoyi* is exclusively lacustrine and Nearctic and may have been so for millions of years (Bousfield 1989; Väinölä and Varvio 1989). In contrast, the *M. relicta* group and the *Monoporeia affinis* group (which comprises the European freshwater pontoporeids) also inhabit circumpolar brackish waters and may have dispersed more recently. No genetic subdivision into sibling species either among European or among American freshwater pontoporeids has been documented, but a counterpart to the divergence among the *M. relicta* group species is found in that between the Fennoscandian *M. affinis* and a so far undescribed North American coastal *Monoporeia* species (Väinölä and Varvio 1989).

Population History in North America

While the independent history of each individual species probably spans several glaciation cycles, the assessment of historical relationships among populations, in the light of paleohydrographical data, will necessarily be limited to the period since the last glacial maximum some 18 kya (Late Wisconsinian/Weichselian/Valdai). On each continent, the populations are chiefly derived from proglacial refugial waters that existed along the continental margins of the major ice sheets. The assessment involves correlating distributional and gene frequency variation patterns with the evolution of connections among lakes (and seas) in the course of the deglaciation. We are specifically concerned with evidence of distinct refugial origins during the last maximum.

In North America, the intricate relationship between the present "relict" crustacean distributions and the late-glacial succession of proglacial lakes that followed the ice front retreating to the north has been illustrated by Ricker (1959) and Dadswell (1974). The dispersal histories were probably similar to those of many freshwater fishes, although more strictly dependent on lentic and downstream connections. The fish fauna in the area once occupied by the Laurentide Ice Sheet is generally divided into elements derived from separate Atlantic, Mississippian, and Beringian freshwater refugial areas (McPhail and Lindsey 1970; Bailey and Smith

1981; Underhill 1986). Of these, the central one may be further subdivided into a Mississippian and a Missourian center of dispersal (e.g., Crossman and McAllister 1986). Separate Atlantic and Mississippian origins have also been postulated for *Mysis* and other "glacial relicts", particularly to explain the present marginal occurrences in the south (Dadswell 1974). Moreover, the records from Waterton Lake, southwestern Alberta (Fig. 2), indicate that even more western refuge(s) must have existed (Ricker 1959; Crossman and McAllister 1986).

The main genetic division of our material into an eastern and a (north)western population group and an apparent intergradation in the Huron basin are also most reasonably explained with colonization by two (or more) separate differentiated refugial stocks. However, with the current reconstructions of drainage history (e.g., Prest 1970; Karrow and Calkin 1985; Teller 1987) the data do not allow an unequivocal localization of the contributing refugia.

In the east, Lakes George (1) and Cayuga (3) were accessible from the earliest proglacial lakes and possible refugia in the Atlantic sector, south of the Hudson River Valley (Glacial Lake Albany) and the Finger Lakes region (Genesee River headwaters; Dadswell 1974). However, associated with ice-front oscillations, a drainage pattern from the Michigan basin (accessible from a Mississippian refugium) east through the Ontario basin and Mohawk and Hudson valleys was already established 13.3 kya, and possibly again about 12.5 kya, before the final deglaciation of the southern Great Lakes basins. On the other hand, the Finger Lakes area also temporarily drained west through the Erie–Huron–Michigan route (e.g., Eschman and Karrow 1985; Teller 1987). In principle, these events could have brought about a mixing of Atlantic and Mississippian stocks before isolation of any of the studied lakes in the eastern area. It may not even be necessary to postulate survival in the Atlantic watersheds, since the eastward drainage from the Michigan area predated the last proglacial lake phase that can account for the present eastern distribution of the "deep-water community" (map 14 in Dadswell 1974).

The central Canadian lakes (8, 9, R), with populations characterizing the western genetic type, are remnants of the vast Glacial Lake Agassiz which covered much of Manitoba and adjacent areas during deglaciation (11.7–8.0 kya). Lake Agassiz may have been accessible (for fish) both from a central Mississippian refugium and from more western Missourian sources (Stewart and Lindsey 1983). A direct access for *Mysis* from the Great Lakes (Superior) northwest to Lake Agassiz area seems unlikely, but migration in the opposite direction would have been possible after 11 kya (although not regarded as an important dispersal route for fish; Underhill 1986). With these connections, the observed east–west genetic division and the intergradation in Lake Huron could be explained with colonizations by differentiated stocks from any two of the Atlantic, Mississippian, and Missourian refugial areas or from all three (when the central Mississippian stock could have been similar to either of the other two or intermediate).

The sample from the Mackenzie Delta area (10) is genetically similar to the Manitoba populations of Lake Agassiz descent. A Beringian or marine origin for this population might not be ruled out: the lake is also inhabited by *Neomysis intermedia*, a species only found in coastal lakes and estuaries (yet the area was not inundated late- or postglacially; Andrews 1989). However, populations higher up in the Mackenzie drainage (Athabasca, Great Slave, etc.) could not have immigrated from the north: in general, the Beringian zoogeographical element did not spread much beyond the Arctic coast, and most of the Mackenzie basin fish fauna is also of Mississippian/Missourian origin (McPhail and Lindsey 1970; Lindsey and McPhail 1986; Bernatchez and Dodson 1991). On the other hand, a direct downstream connection from Glacial Lake Agassiz north to the Mackenzie drainage area is also regarded as unlikely (Schreiner 1983; but cf. Klassen 1989). The faunal exchange was probably through Glacial Lake Peace, upstream to the west (Lindsey and McPhail 1986), and *Mysis* has actually also been reported from Lesser Slave Lake in that area (Fig. 2; Ricker 1959). If the westerly route existed, the Mackenzie drainage populations should provide further evidence for a separate southwestern refuge. This would also have contributed to the Agassiz stock, in accord with the genetic data.

Returning to the east, drainage history may also explain aspects of the differentiation recorded below the main genetic subdivision. An apparently western genetic component in the southeasterly Lake Champlain population (2; see Fig. 4) can be explained by a western gene flow that reached the Champlain basin only after the isolation of the other eastern lakes. Lake George (1) was isolated from waters in the Champlain Valley approximately 13 kya (Connally and Sirkin 1973) and both Cayuga and Mazinaw (3, 5) from the Ontario basin (Glacial Lake Iroquois) 12.0 kya (Dadswell 1974; Muller and Prest 1985). Subsequently, the brackish Champlain Sea penetrated into the St. Lawrence Valley about 11.8–8.5 kya, extending to the Lake Champlain area until its final isolation as an independent lake 10.0 kya. This marine influence probably initially extirpated the resident "relict" populations of the earlier freshwater phase in the basin (Dadswell 1974).

From 10.9 kya, Lake Agassiz, which earlier discharged to the Mississippi system, started to drain eastward towards the Champlain Sea, where the voluminous freshwater input caused a change to a lower-salinity environment (Teller 1985, 1987). From 10.5 kya until the isolation of Lake Champlain, this drainage via the Upper Great Lakes was directly through the Ottawa River valley to the Champlain Sea, bypassing the present Erie–Ontario route. It is plausible that a substantial proportion of recolonizers of the Champlain Valley during the more hospitable brackish phase, until the isolation of Lake Champlain, descended from Upper Great Lakes populations (including Agassiz), while eastern elements (e.g., from the Ontario basin or Lake George, unaffected by the Champlain Sea and Lake Agassiz input) still had a major influence. This could account for the mixed character of the Lake Champlain *Mysis* population.

There are also considerable differences among the eastern populations which are not readily attributable to the mixing of a few ancestral stocks but more likely reflect more recent population bottlenecks. Three of the four oldest populations (1, 3, 4) are similar, suggesting a homogeneous population in the eastern area some 12 kya, but that of Lake Mazinaw (5) exhibits deviations most reasonably attributed to drift (e.g., monomorphism at *Gpi* and *Mpi*). Lake Mazinaw was only briefly and marginally connected to Lake Iroquois before its final isolation (Muller and Prest 1985).

Although the North American data are most plausibly interpreted in terms of (at least) two distinct late-glacial

refugial origins, they do not warrant further taxonomic subdivision of the North American freshwater *Mysis*. No indications of noninterbreeding (heterozygote deficiencies, interlocus associations) were seen in the genotypic structure of samples of possibly mixed origin (i.e., sites 2 and 7). Likewise, similar patterns of gene frequency variation observed in several fish species and thought to reflect differential refugial stocks have not been given formal taxonomic significance (e.g., Seeb et al. 1987; Ihssen et al. 1988; Ward et al. 1989). Compared with the well-documented differentiation within the lake whitefish *C. clupeaformis*, the differentiation in *Mysis* actually represents a narrower geographical scale: the total continental range of *M. relicta* sp. IV corresponds to that of a single one of the four principal conspecific North American phylogeographic assemblages or refugial races of the *C. clupeaformis* complex (Bernatchez and Dodson 1991; Bodaly et al. 1991, 1992).

By contrast, Bousfield (1989) has suggested, on morphological grounds, that the "glacial relict" amphipod *D. hoyi* comprises several distinct species in North America. This remains controversial: allozyme data did not indicate any systematic distinction between two sympatric morphs of *Diporeia* suspected by Bousfield of being of subgeneric rank (Väinölä and Varvio 1989). The pattern and level of intraspecific allozyme differentiation among four eastern geographical populations of *D. hoyi* (F_{ST} = 0.06) were similar to those of *Mysis* of the same lakes (F_{ST} = 0.09).

Population History in Northern Europe

Background

Segerstråle's (1957, 1982) model of the immigration of the "relict" crustaceans to their present northwest European range involved dispersal from a late-glacial ice lake in the White Sea region to the southern Baltic Sea area in ice-marginal continental waters. This hypothesis contrasted with earlier views of a marine immigration to the Baltic during a Late Weichselian interstadial phase. Both models assumed subsequent sluicing-up with a glacier readvance to explain the colonization of the southernmost continental occurrences. As Segerstråle still advocated a relatively recent descent of the freshwater species from marine ancestors in central Siberia, the invasion of Europe was dated to the latest glaciation(s). Newer paleogeographical research has added to the evidence of effective Late Weichselian continental east-to-west dispersal routes (Kvasov 1979; Grosswald 1980).

On the other hand, the idea of a recent marine ancestry has been refuted (Holmquist 1966; Väinölä 1986), and the existence of distinct species within the morphological *M. relicta* indicates that effective long-term population isolation has taken place during the (early) history of the freshwater lineage. The first records of the sibling species suggested different postglacial dispersal histories and ecological preferences for them (Väinölä 1986). Differential salinity-related distributions of spp. I and II within the Baltic and the only freshwater record of sp. II, from a lake isolated from a late (4 kya) phase of the basin, raised the hypothesis that sp. II might have invaded the Baltic only after the establishment of the final marine connection, from the west. In considering the evidence for different refugial origins and dispersal histories below, a primary issue is again whether the presence of a species (or an intraspecific stock) in an area can be indicated before establishment of certain dispersal connections. With the hypothesis of differential euryhalinity of spp. I and II, the environmental history of the Baltic is of particular interest, as the basin underwent alternating fresh- and brackish-water phases due to a complex sequence of connections to the western sea (Kattegat). In addition to the data on the *Mysis* spp. themselves, the distributional correlations with other "relict" taxa are examined; some of these species (*Pallasea quadrispinosa*, *Gammaracanthus lacustris*) have narrower salinity requirements than *Mysis* spp. and may thus provide additional insight to past environments.

Eastern and southeastern populations

The East Karelian records (14, 15, Lake Onega) indicate that both spp. I and II inhabited eastern continental refuges. At the last glacial maximum, several proglacial lake systems existed in northwestern Russia, draining south to the Caspian and Black seas (Fig. 1). In the course of deglaciation, these waters later joined a common westward drainage pattern along the glacier margin (e.g., Kvasov 1979; Grosswald 1980; Faustova 1984), but there was no access from the Baltic (or south of the Baltic) northeast to the Lake Onega – White Sea area at any stage. The White Sea and Lake Onega basins were accessible from the same eastern refuges (the Upper Volga and North Dvina systems), and the two basins had temporary connections until approximately 10 kya (Kvasov 1979). Lake Paanajärvi (15, sp. II) was only deglaciated and isolated after 9.9 kya, when a direct marine connection to the White Sea basin had been established (Hyvärinen 1973; Kvasov 1979). Environmental salinity might therefore have impeded the dispersal of sp. I in this direction (cf. below). However, the presence of the stenohaline *P. quadrispinosa* in the lake does not accord with this hypothesis. Drainage of West Russian waters southwest towards the North Sea basin was probably established 14–13.5 kya (Grosswald 1980).

There may also have been other sources of dispersal. Ice-marginal waters in Poland flowed west through the glacial maximum; some proglacial lakes at the headwaters of Dnepr in Belarus, initially draining south, also shifted to a westward flow early during the deglaciation, before the deposition of the Pomeranian/Vepsovo stage moraines approximately 15 kya. Most of the southern continental *Mysis* lakes (e.g., those in Poland and Germany, with sp. I) lie close to this moraine zone and were probably isolated shortly after ice retreat from this position, before the final western redirection of the West Russian drainages (Kvasov 1979; Grosswald 1980). The populations in these lakes could plausibly be derived from the southerly proglacial lakes — as regards some *M. relicta* group populations in Belarus, Lithuania, and Latvia (Fig. 1), most probably from the Dnepr headwater refuge. These occurrences have earlier been attributed to sluicing-up with glacier readvances following temporary retreats beyond the stadial moraine zones (enabling intermittent immigration from the east) (e.g., Segerstråle 1957; Müller 1964); it is unclear whether such oscillations were great enough to account for the whole southern distribution.

Species I in the Baltic area

By the time deglaciation reached the southern Baltic (approximately 13 kya), most proglacial waters had attained a westward drainage through this area to the Kattegat and the North Sea. The Baltic Ice Lake was established in the basin some 12 kya (Kvasov 1979). As the ice had receded to the central part of the basin, a direct marine connection across central Sweden (between sites 6–8 and 10, Fig. 1) opened for

a short period about 11.0 kya, and after a brief redamming of the Baltic Ice Lake by the Younger Dryas readvance, reopened about 10.4 kya (Björck and Digerfeldt 1989). This initiated the Yoldia (Sea) phase in the Baltic, which lasted some 1000 yr, and was followed by yet another lacustrine stage, the Ancylus Lake. Our material includes populations isolated from Baltic waters during all these phases. Norra Bjärken and Stora Sinnern in eastern Sweden were isolated at about 11.5 kya from the Baltic Ice Lake (Svensson 1989). A few lakes both in eastern Sweden and southeastern Finland were isolated in connection with the second drainage of Baltic Ice Lake to sea level (e.g., Eronen 1983; Svensson 1989). And most of the Finnish lakes and many of those studied in Sweden arose as a result of strong isostatic rebound during the Yoldia and subsequent Ancylus phases, until 8.5 kya (Agrell 1979; Eronen 1983; see Väinölä 1986). Although both species should have had a similar access from the Lake Onega direction to the Baltic, only sp. I has so far been found in these lakes isolated directly from the Baltic during its early history.

The final establishment of a marine connection through the Danish straits (8.5 kya) was followed by the Litorina Sea phase of the Baltic (until 4 kya), with a salinity somewhat higher than the present (Kvasov 1979; Eronen 1983). Most lakes isolated at this time or later are in central eastern Sweden and include Lake Båven (approximately 4 kya), where spp. I and II coexist. The youngest lake studied, Mälaren (sp. I), was isolated some 1 kya (Miller 1982).

There is, therefore, evidence for sp. I occurring widely in the continental ice-marginal waters and in the Baltic through its late- and postglacial history. Overall, the spread of sp. I probably largely followed the previously established model of "relict" dispersal in Fennoscandia, to and through the Baltic waters (Segerstråle 1957). This includes a westward migration through the central Swedish connection to the Vänern basin and further to southeastern Norway, as far as Lake Mjøsa — the invasion was obviously completed soon after deglaciation reached Mjøsa about 9.4 kya (data on Norwegian distribution in Mathisen (1953) and on shoreline displacement in Sørensen (1983) and Hafsten (1983)). Species I coexists with most of the other "relict" crustaceans, including *G. lacustris* and *P. quadrispinosa*, in the main parts of their ranges; *P. quadrispinosa* always accompanies sp. I, except in the open Baltic. A common late-glacial freshwater history for these species can be inferred.

Gene frequency differences within sp. I are small and mostly attributable to postcolonization changes (overall, $F_{ST} = 0.05$; R. Väinölä, unpublished data). The populations in the Baltic show a slightly closer affinity to eastern (Karelian) than to southern continental populations. Some of the other "relict" species, e.g., *G. lacustris*, are absent from the southern continental area, which also supports a direct immigration from the east to the Baltic basin (cf. Segerstråle 1957).

Species II in southern Scandinavia and the Baltic

Species II accounts for many of those southwestern occurrences of the *M. relicta* species group which have been difficult to reconcile with the above (Segerstråle's) dispersal scheme and thus reinforces the model for sp. I. As regards sp. II itself, the working hypothesis of a purely western origin is refuted by the Karelian records. The question remains whether colonization was exclusively from the east or whether separate western refuges also existed. Another question concerns the background of the differential distributions of the two species despite similar dispersal opportunities, e.g., the absence of sp. II from the older Baltic lakes. We shall first consider the Scandinavian–Baltic area from various points of view and then the Irish populations separately.

The western Swedish sp. II populations date back to the time of the direct Baltic – North Sea connection at the Yoldia time. Lake Öresjön (6; 76 m asl) and the uppermost *Mysis* lake in the drainage comprising sites 7 and 8 (Lake Anten, 66 m) were isolated approximately 10.1–10.2 kya (Dennegård 1984; Björck and Digerfeldt 1991), at a time of strong outflow from the Baltic through the area, but with a record of a marine fauna in the surrounding waters (Fredén 1988). Lake Aspen (8; 14 m asl) was probably secondarily colonized from the upstream populations since it was isolated from the sea later during the oceanic period, when *Mysis* would hardly have survived in the area (cf. Jägerskiöld 1912). Lake Stokkelandsvatn in southwest Norway (5; 18 m asl) lies close to the marine limit, but may have been briefly inundated by a Younger Dryas transgression around 11.0–10.4 kya (Anundsen 1985), i.e., well before the deglaciation of the southeast Norwegian sp. I lakes.

The Danish Furesø population (9) is probably older. The area was not inundated by marine or Baltic waters, and the actual mode of colonization remains unclear. The lake was finally deglaciated and isolated some 13 kya (Lundqvist 1986). The fauna could have been derived directly from ice-marginal continental refuge populations or by sluicing-up with a glacier oscillation from the present Öresund area (the Low Baltic/Belt Sea advance, e.g., Segerstråle 1957). Immigration from the east should have been possible by this time (Kvasov 1979).

Thus, considering the ages of populations in relation to the hydrographical connections, a separate western origin for the central sp. II populations cannot be confirmed or refuted on distributional grounds. Gene frequencies do not solve the question either. The most divergent genetic structure in Fennoscandia, in Lake Stokkelandsvatn (5; see Fig. 3a), is most reasonably attributed to a postglacial population bottleneck in the colonization phase or later. This is evident from the genetic features (monomorphism at most loci) and is consistent with the probable population history (dispersal through a saline route to a remote, small lake (0.2 km^2)). Among the remaining populations (the eastern group; Fig. 3b), the similarity of the Baltic and western Swedish populations suggests that their common composition already prevailed 10 kya, when the western populations were isolated. The timing and direction of the spread of this composition remain unsolved, however. With the paleohydrographical background, the differences between the two west Swedish drainages (6, 7) and between the Baltic and Lake Båven populations (12, 10) can be attributed to post-isolation changes since 10 and 4 kya, respectively. These changes typically involve a (near) loss of some rare but widespread alleles in one population or the other. The Karelian populations (14, 15) are not more divergent in quantitative terms (genetic distances) than the populations just discussed are from each other (Fig. 3b). Nevertheless, some qualitative features in the (common) Karelian allozyme profile, including both presences (*Pgd*, *Pgm*) and absences of allozymes (*Pep*, *Tpi*), may point to a more distant relation-

ship (Table 3). More data on the eastern populations are needed, however, to rule out local, recent phenomena as a cause of these differences.

Whatever the refugial history of sp. II, the lack of evidence for its presence in the early Baltic stages remains problematic. If it did not reach the early Baltic Ice Lake (>11 kya) from the east, it should still have been able to enter from the west during the Yoldia phase, as some Arctic marine animals temporarily did (e.g., Fredén 1988). On the other hand, the connections would have allowed an early westward spread of sp. I to the southwestern coastal lakes now only inhabited by sp. II. To account for the distributional differences of spp. I and II, differential environmental tolerances and interspecific interactions may both have to be invoked.

A broader euryhalinity of sp. II was first suggested by the current distributions within the Baltic Sea, where this species is usually exclusive in salinities >6.5‰ (Väinölä 1986; the total distribution is limited by approximately 12‰ bottom salinity in the south). In the paleohydrographical framework (see above), the lacustrine distributions in western Sweden provide further evidence for this hypothesis. The dispersal of sp. I has been truncated immediately southwest of Lake Vänern, while sp. II has survived in lakes closer to the coast, isolated during the marine influence. Notably, *P. quadrispinosa* and *G. lacustris* are also absent from the Swedish west coast lakes whereas the more euryhaline "relict" crustaceans *Limnocalanus macrurus*, *Monoporeia affinis*, and *Saduria entomon* do coexist with sp. II there (Holmquist 1966). Environmental conditions thus actually seem to have been critical in determining the distributions, sometimes allowing spread of sp. II while preventing that of the more stenohaline sp. I, *P. quadrispinosa*, and *G. lacustris*. The latter three taxa could disperse in the western area later, after the effective isolation of the Vänern basin from the Kattegat waters (Fredén 1988; Björck and Digerfeldt 1991). In contrast with the lakes in western Sweden, the fauna of the Danish Lake Furesø (9) does include both sp. II and *P. quadrispinosa* (which is also present in other Danish lakes); the immigration to this region thus probably did not involve saline environments.

Upper salinity limits do not explain the absence of sp. II from the lakes isolated from the early (freshwater) phases of the Baltic, however. A hypothesis of competition from sp. I effectively preventing colonization by sp. II of lakes in the process of isolation could account for this feature. Despite the inherent success of both species in fresh and brackish environments, cases of lacustrine coexistence are rare. Also, in Baltic coastal gradients, sp. II is generally absent from the nearshore localities with salinities <4–5‰, where sp. I prevails (Väinölä 1986 and unpublished data). In combination, the salinity and competition hypotheses predict that colonization by sp. II is facilitated in higher salinities through the removal of the competition factor (as implicated for the Swedish west coast area above). Such conditions probably were met in the Baltic during the Litorina period 7–4 kya; actually, the higher salinity in those times has been suggested to have temporarily exterminated the "relict" crustaceans from all but the most peripheral parts of the Baltic basin (Segerstråle 1957). It is noteworthy that at the single sympatric lacustrine site in the Baltic area (Båven), which dates back to the late Litorina time, the sp. I population is strongly differentiated from its conspecifics in the Baltic and neighboring lakes (F_{ST} = 0.16), suggesting a severe population bottleneck (R. Väinölä, unpublished data).

However, in a survey of several additional lakes isolated during or after the Litorina phase, we failed to demonstrate a more general effect of salinity on the composition of "relict" fauna in the Baltic basin lakes (e.g., by facilitating colonization by sp. II). No further records of sp. II were made, although sp. I was found in several such lakes (Lappajärvi in Finland; Storsjön, Yngaren, Hunn, and Mälaren in eastern Sweden), and in two Litorina lakes lacking *Mysis* altogether, other "relict" species were still present (Klämmingen and Yngern, in the vicinity of Båven). Even *P. quadrispinosa* was found in all these lakes.

Species II in Ireland

The Irish *Mysis* populations probably represent a separate refugial origin within sp. II. The differentiation of this group (Fig. 3a) is similar in magnitude to that between the postulated North American refugial stocks (Fig. 4), and there is no reduction of heterozygosity to suggest a recent bottleneck (unlike in the similarly distinct Lake Stokkelandsvatn population (5)). The differentiation is mainly a function of a single locus (*Pep-2*), however, and the genetic evidence therefore not very strong. More importantly, a close relationship between the Fennoscandian and Irish sp. II seems unlikely on geological grounds. A Late Weichselian ice-marginal dispersal route from the eastern continental area through the North Sea region to the English Channel and Irish coasts (Segerstråle 1982; Grosswald 1980) is not supported by recent reconstructions: the continental ice-marginal waters more probably discharged from the North Sea directly north towards the (unglaciated) Norwegian Sea (e.g., Sejrup et al. 1987), and the Channel only opened approximately 8.5 kya.

The local colonization history of the Irish lakes and the nature of possible refugial habitats are poorly understood. There is no evidence of suitable refugial glacial lakes in the now emergent parts of Ireland. In any case, the Irish Sea probably acted as a dispersal center during the deglaciation. It has been suggested that the basin was for some time occupied by a large ice-dammed lake, bounded by ice to the north and by a temporary land bridge between southeastern Ireland and Cornwall (southwestern England) to the south (e.g., Synge 1985); this idea is not generally accepted, but the meltwater input and narrow connections to the sea undoubtedly gave the embayment a brackish-water character at that time (e.g., Eyles and McCabe 1989).

The colonization of the single *Mysis* lake in England, Ennerdale Water in Cumbria (121 m asl; the population may now be extinct), has been explained by damming-up of a temporary lake by a glacier readvance or a minor oscillation during the deglaciation of the Irish Sea (Tattersall 1942; Fryer 1981; but cf. Huddart 1991). A sluicing-up event has also been postulated for the Lough Neagh population (4; 15 m asl) (Charlesworth 1928), but this basin probably also had a direct connection to the Irish Sea (Synge 1985; Eyles and McCabe 1989). The other Irish populations (33–44 m asl), now in catchment areas draining to the west, may have been accessed from the Irish Sea basin across the low-lying Central Plain of Ireland through temporary connections created by meltwater in the downwarped ice-marginal terrain during the deglaciation — recent interpretations suggest considerably higher relative sea levels at that phase than was earlier believed (Eyles and McCabe

1991). At any rate, no reliable reconstructions of the relevant Irish hydrography at the deglaciation exist.

Other "relict" crustaceans are not present in Ireland, but *L. macrurus* is known from Ennerdale Water, and the distributions of some originally anadromous fish in the British Isles correlate with that of *Mysis*. Particularly, *C. autumnalis*, which is widespread in Siberia and North America, is present in the Irish *Mysis* lakes but not elsewhere in northwestern Europe west of the White Sea (Maitland 1970; Bodaly et al. 1991). Ferguson et al. (1978) suggested that *C. autumnalis* immigrated along a marine route during the last glaciation. The evidence at hand is insufficient for arguing for or against a common history of these species.

Summary for northern Europe

A genetic and paleohydrographic analysis indicates both inter- and intraspecific refugial disjunctions for the North European *M. relicta* group populations. The single subarctic population of sp. III bears no zoogeographical relationship to the classical boreal "relict" element, but may rather be related to the *M. relicta* group populations of Arctic estuaries and archipelagoes. The Irish *Mysis* populations, also disjunct from the main North European range, are conspecific with the Fennoscandian sp. II, but probably represent another local refugial stock distinct since the last glacial maximum.

The populations in the Fennoscandian main range involve two overlapping species, both widely distributed from Karelia to southern Norway, and also present in the Baltic Sea. Both spp. I and II occupied eastern freshwater environments in northwestern Russia at the peak glaciation. Additional, more westerly refuges may have existed, but the evidence remains equivocal. In any case, despite the fact that hydrography should have granted similar opportunities for spread of both species from the eastern sources over their composite Fennoscandian range, the current distributions are conspicuously different (Fig. 1). This late-glacial distributional differentiation is partly attributable to the differential relationships of the two species to environmental salinity.

Species I has been present in the Baltic since the early deglaciation of the basin. The distribution of this species largely coincides with those of other stenohaline "relict" taxa, and its dispersal essentially followed the model outlined by Segerstråle (1957). The localities only accessible through relatively saline routes are accounted for by spp. II and III. Particularly, the distributional patterns of spp. I and II in coastal lakes of southwestern Scandinavia, as related to the evolution of the temporary direct Baltic–Kattegat connection in the Yoldia time (around 10 kya), illustrate a critical role of environment in determining the distributions. This is supported by distributional correlations with the other "relicts" and by the current distributions of spp. I and II in Baltic salinity gradients, which all indicate a broader euryhalinity for sp. II than sp. I. Neither this difference nor a potential refugial disjunction explains the lack of any evidence of sp. II in lakes isolated from the early Baltic phases, prior to some 4 kya. This pattern could be due to competitive interactions of the two species, which seldom coexist in lakes.

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Spatial Distribution of Pacific Halibut (*Hippoglossus stenolepis*): An Application of Geostatistics to Longline Survey Data

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The spatial distribution of Pacific halibut (*Hippoglossus stenolepis*) in the Gulf of Alaska was analyzed using longline catch per unit of effort (CPUE) data collected during three grid surveys in 1984, 1985, and 1986. Geostatistical techniques were used: (i) a variographic analysis to model and estimate the spatial structure of halibut abundance and (ii) ordinary kriging to predict local abundance. Available small-scale information made it possible to model satisfactorily the spatial structure. Results show (i) a persistent large-scale east–west difference in average CPUE and (ii) spatially correlated CPUE data with an average covariance decreasing as the distance between observations increased, over a range of 0–20 nautical miles (nmi) in 1984 and 1985, and 50 nmi in 1986. The survey design had limitations in that it was too unbalanced, with stations very close together along north–south transects, and transects too far apart from each other. Consequently, prediction error was small close to the transects and large in between in a clear banded pattern. To achieve a more regular coverage of the same area, a new survey design was developed: the global variances obtained with this new design using the variogram parameters for 1985 and 1986 were 20% lower than those based on the old design.

Grâce à des données de captures par unité d'effort (CPUE) obtenues lors de campagnes annuelles en 1984, 1985 et 1986 pour un ensemble de stations prédéterminées, nous avons pu analyser la distribution spatiale du flétan du Pacifique (*Hippoglossus stenolepis*) dans l'une des principales zones de pêche, située dans le Golfe d'Alaska. Des techniques géostatistiques ont été appliquées : i) une analyse variographique afin d'estimer et de modéliser la structure spatiale de l'abondance du flétan et ii) un krigeage ordinaire pour prédire l'abondance locale. L'existence d'information à petite échelle a permis de modéliser correctement la structure spatiale via un changement de support. Les résultats montrent i) à grande échelle, une différence persistante entre les CPUE à l'est et à l'ouest de la zone et ii) que les CPUE sont corrélées dans l'espace, avec une covariance moyenne qui décroît avec la distance entre les observations, et ce sur une portée d'environ 20 miles nautiques (mn) en 1984 et 1985 et 50 mn en 1986. Le protocole d'échantillonnage comportait des limitations liées à son asymétrie, les transects étant trop éloignés les uns des autres, tandis que les stations d'un même transect étaient très proches. Ceci entraîne une erreur de prédiction faible près des transects, mais parfois importante ailleurs. Une nouvelle grille d'échantillonnage est définie afin d'obtenir une couverture plus régulière de la zone. Les tests réalisés montrent un gain de variance globale de l'ordre de 20 % si cette grille avait été utilisée au lieu de la grille originale en 1985 et 1986.

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The analysis of the spatial distribution of marine populations has aroused a great deal of interest in recent years. In fisheries, this is mainly motivated by the fact that ignoring spatial patterns when using commercial catch per unit of effort (CPUE) data may lead to unreliable abundance indices for exploited populations, with subsequent consequences for stock assessment. As the spatial allocation of fishing effort may depend on the distribution of fish abundance, abundance maps (e.g., obtained from survey data and possibly combined with commercial data) may be necessary to avoid the bias inherent in classical CPUE indices computed ignoring spatial considerations (Beverton and Holt 1957, section 10). Among the possible statistical tools for describing spatial distributions of marine populations, geostatistical techniques (Matheron 1965) appear to be particularly suitable because they explicitly account for spatial correlations (Rossi et al. 1991). These techniques were first introduced in fisheries science by Conan (1985) for estimating the abundance of a shellfish population. Since then, a number of applications have dealt with acoustic survey data, either in a mapping perspective (Simard et al. 1992) or for the estimation of global abundance (Anonymous 1991; Sullivan 1991; Guillard et al. 1992; Petitgas 1993). Indeed, data obtained from acoustic techniques are sampled almost continuously along transects; they are thus obviously spatially correlated and therefore a geostatistical approach is highly recommended. Geostatistics have also been used to analyze nonacoustic survey data (Conan and Wade 1989; Petitgas and Poulard 1989; Freire et al. 1992; González-Gurriarán et al. 1993), but these applications are

relatively less common. This is in part because many surveys follow random sampling schemes, which are suboptimal for a geostatistical approach, while they deliberately satisfy the assumptions of random sampling theory.

Most applications of geostatistical techniques comprise two steps: (i) the identification of the spatial structure through the modelling and estimation of the variogram and (ii) the prediction of abundance from kriging, either locally for mapping, or over the whole area for global estimation. In the literature mentioned above, there exist generally converging concerns regarding the techniques applied. First, kriging predictions are very sensitive to the modelled variogram. Therefore, the analysis of the spatial dependence of the data, i.e., the variographic analysis, is central to the results. However, the parameters of the variogram model are most of the time estimated in a rather subjective way, namely visually. This is of no consequence if the empirical variogram is very informative, particularly near the origin. For instance, in the case of acoustic survey data, one can expect substantial information at small scales because of the way data are collected. Other types of surveys may pose a problem in this respect because, due to sampling costs, there is a trade-off between the size of the surveyed area and the average distance between stations. Accordingly, another important issue addressed in the literature is that of sampling design. Applying geostatistics does not require a special sampling design, but a regular placement of stations may greatly improve results in terms of prediction error (Cressie 1991). Information about the spatial dependence of the observations can be used to evaluate the efficiency of alternative sampling plans for geostatistical analysis. Schotko and O'Keeffe (1990), for example, compared different fixed and random designs for the case of an insect population. Other common difficulties in the application of geostatistical techniques result from nonstationarity and anisotropy problems.

In this paper, we apply geostatistics to analyze the spatial distribution of Pacific halibut (*Hippoglossus stenolepis*) using longline survey data. We were interested in estimating maps of abundance for an area including major fishing grounds in Alaska. Some of the problems mentioned previously were encountered in our analysis. First, nonstationarity was identified and dealt with by stratifying the area into two regions. Second, the initial observational scale, namely catch counted per longline, was found to be inadequate for estimating the variogram parameters. A change of support (Cressie 1991) to a smaller scale resulted in substantial improvement. Both local and global abundances were then predicted from ordinary kriging, with associated prediction errors. Finally, a new survey design was tested and found to substantially decrease the prediction errors.

Survey Data

Pacific halibut are found on the continental shelf of the North Pacific Ocean, from California to the Bering Sea, and down the Asian coast; largest concentrations occur in the Gulf of Alaska. From 1963 to 1966 and from 1976 to 1986, the International Pacific Halibut Commission (IPHC) conducted a series of surveys of the major fishing grounds using longline gear. The surveys were always conducted during summer months, when halibut are on shallower banks and coastal waters; this is also the season when the commercial fishery takes place. The analysis presented in this paper focused exclusively on the last three surveys conducted in the area around Kodiak Island, Alaska, during July 1984, May–June 1985, and July–August 1986. Earlier years were not considered because a different hook type was used in those surveys, so results are not comparable.

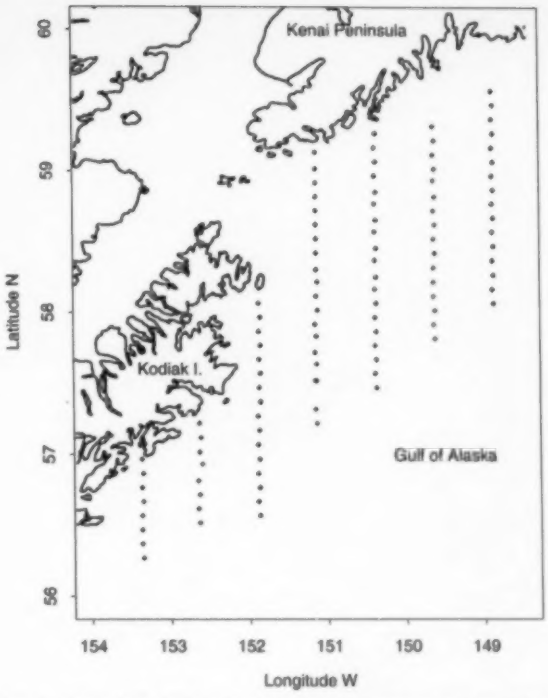

FIG. 1. Sampling design of the longline surveys conducted by IPHC in 1984, 1985, and 1986 in the area off Kodiak Island, Gulf of Alaska.

The survey design consisted of approximately 100 stations (depending on the year), distributed along seven north–south transects running outward from the shoreline to a depth of approximately 350 m (Fig. 1). The transects were 24 nautical miles (nmi) apart, and the distance between adjacent stations was 6 nmi. Each survey took approximately 1 mo to complete. At each station, a longline was set in a north–south direction. Each longline consisted of several contiguous units of gear called skates. A station is thus referred to in the following as a set (of skates). In 1984, each set consisted of a string of eight 1500-ft skates (total length of a set approximately 2 nmi) whereas in 1985 and 1986, there were six 1800-ft skates per set (total length of a set approximately 1.8 nmi). In every case, each skate contained 75 circle-hooks baited with salmon or herring on alternate hooks; hook spacing was thus approximately 20 ft in 1984 and 24 ft in 1985 and 1986. This year-to-year difference in hook spacing could not account for more than a 10% difference in CPUE according to the standardization algorithm currently used by IPHC (P. Sullivan, personal communication). On a regular fishing day, four sets were deployed in the morning, and the gear was allowed to soak for a period of 5–12 h before being retrieved; the shortest soak corresponded to the first set retrieved and the longest to the last set of the day.

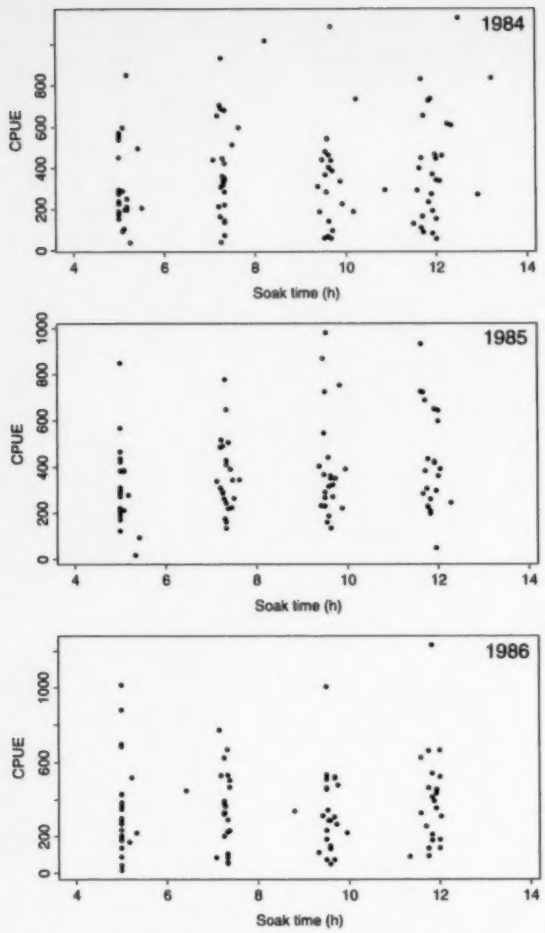

FIG. 2. Average catch per skate (CPUE, net weight in pounds) as a function of soak time at each set in the surveys of 1984, 1985, and 1986.

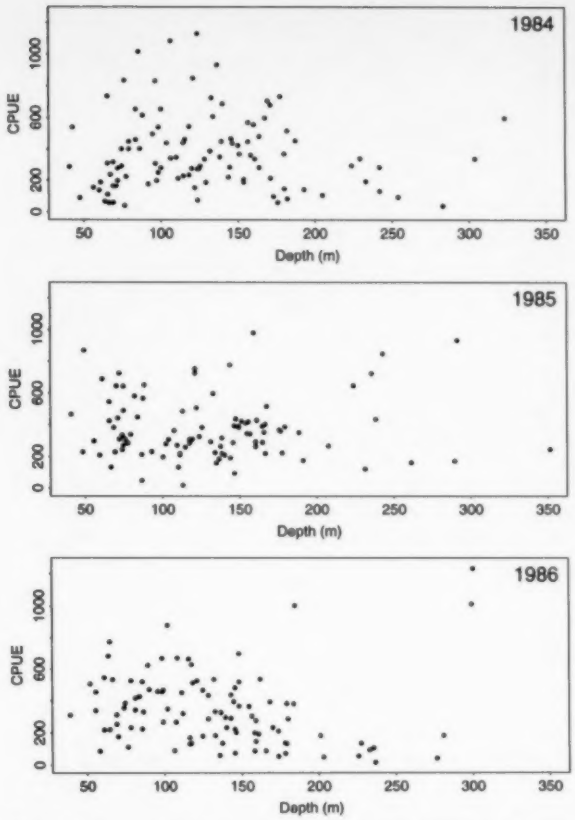

FIG. 3. Average catch per skate (CPUE) as a function of mean depth at each set in the surveys of 1984, 1985, and 1986.

The size of all halibut caught on each skate within each set was recorded. Total catch of halibut in net weight for each skate was computed using the length–weight relationship in Clark (1992). In addition, depth and numbers of other species in the catch were recorded for each set. The CPUE considered is the average catch per skate in a set. In order for the survey results to be comparable with the commercial CPUE index used by halibut fishermen and IPHC, catch was expressed in weight (pounds) and only fish longer than 81 cm (the legal commercial size) were included in its computation.

Preliminary Data Analysis

Analyzing fish populations from CPUE data presumes a close relationship between CPUE and fish density, which is commonly assumed for stock assessment. For instance, an estimate of the average CPUE for the commercial longline fleet is currently used by IPHC as an index of abundance of Pacific halibut. Support for this assumption comes from the fact that historical trends in commercial CPUE appear to track fairly well the time series of estimates of total exploitable biomass obtained independently of CPUE using catch-at-age data (Parma 1993). In this analysis, the assumption is required locally, i.e., at each station the CPUE is closely related to local halibut density. In addition, this relationship has to be consistent from station to station. As the gear used comprises a limited number of hooks, there are two important factors likely to affect this relationship: (i) the effect of varying soak time and (ii) the abundance of other species competing for bait with halibut, which may change from station to station and from year to year. Addressing these problems exceeds the scope of this paper. However, we conducted some exploratory analysis to check if any related effect could be detected from the CPUE data. Regarding (i), no statistically significant trend between CPUE and soak time was found in any of the three years when data were analyzed independently of location (t-test, $P > 0.05$) (Fig. 2). With respect to (ii), the effect of other species competing for bait with halibut could be more of a concern a priori. However, the proportion of halibut in the total catch is high in the area off Kodiak (62% on average) and the proportion of "successful" hooks, i.e., a hook with nonzero catch is low (25% on average). These two facts seem to indicate that the competition problem is not likely to generate major artifacts in this area.

CPUE did not show any clear dependence on depth, at least in the depth range more intensely covered in the survey,

TABLE 1. Average catch (wet weight in pounds) per skate in the western and eastern parts of the Kodiak area.

	1984	1985	1986
West	400	419	484
East	361	358	293

i.e., on the feeding grounds (20–200 m) (Fig. 3). With the exception of one or two stations, very few fish were caught beyond 200 m in 1984 and 1986 (only a few deep stations were fished, however). Relatively high catches were still obtained at larger depths in 1985, probably in relation to the earlier scheduling of the survey that year. These few points may reflect the unfinished spring migration of halibut from the deep winter spawning grounds to the shallower summer feeding grounds. However, the lack of contrast in depth in the data prevents the drawing of any conclusions regarding a general relationship between CPUE and depth.

Another feature of the data is the consistent dissimilarity between the CPUE in the western and eastern parts of the area (Table 1), CPUE always being higher in the western part, i.e., west of longitude 151°30′W (limit defined by eye). The east–west limit approximately separates two important fishing grounds, Albatross Bank and Portlock Bank (Fig. 4). Although we were not able to link this pattern with any known environmental features, allowing for these areal differences was helpful for analyzing the spatial structure of halibut abundance.

Estimating and Modelling the Spatial Structure: Variographic Analysis

Let $S(\mathbf{x})$ denote a random variable corresponding to halibut abundance (density) at location $\mathbf{x}$ where $\mathbf{x}$ is defined by latitude and longitude in a two-dimensional space. This spatial process was observed at g stations of a survey grid, yielding a set of observations $\{Z(\mathbf{x}_i), i = 1, \ldots, g\}$. We assume that (i) the observations $Z(\mathbf{x})$ and the spatial process $S(\mathbf{x})$ differ only through white-noise measurement error and (ii) the spatial distribution of halibut was stable throughout the survey (approximately 1 mo), so that all CPUE observations reflect the same underlying spatial process. The latter is a rather strong assumption for fish populations in general, and which is not particular to geostatistical applications. Although fish movement may be a problem in this regard, the distribution of halibut over the feeding grounds in summer is believed to be stable, at least at a relatively broad spatial scale.

The true process $S(\mathbf{x})$ can itself be decomposed into a large-scale deterministic component $\mu(\mathbf{x})$ and a small-scale stochastic component $\epsilon(\mathbf{x})$ with zero mean:

$$S(\mathbf{x}) = \mu(\mathbf{x}) + \epsilon(\mathbf{x}). \quad (1)$$

Modelling local halibut abundance $S(\mathbf{x})$ thus requires handling the stochastic component $\epsilon(\mathbf{x})$. Commonly used approaches attempt to explain $S(\mathbf{x})$ as a function of exogenous variables (like depth, for instance) by modelling a spatial trend in the deterministic component $\mu(\mathbf{x})$ while assuming that the $\epsilon(\mathbf{x})$ are not correlated (although this is not a requirement). Under this assumption, it is sufficient to predict $\mu(\mathbf{x})$

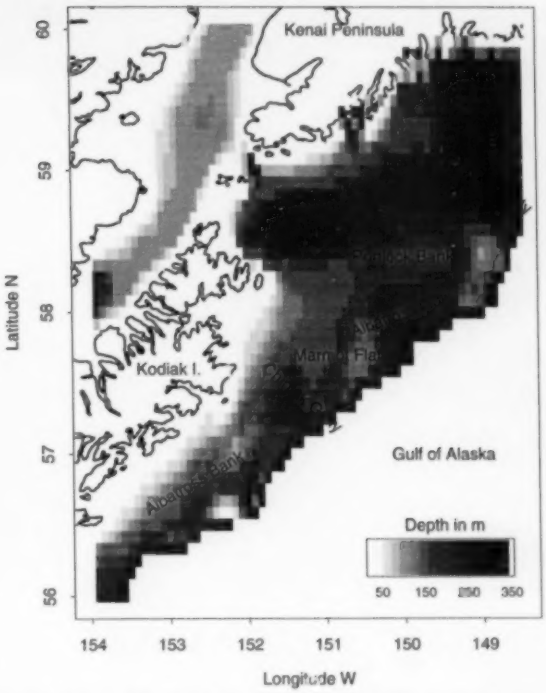

Fig. 4. Map of depth for the survey area (data source: National Oceanic and Atmospheric Administration, U.S.).

to obtain an accurate picture of the process $S(\mathbf{x})$ (e.g., see Swartzman et al. 1992). In the geostatistical approach, instead, one is rather interested in modelling and *predicting* $\epsilon(\mathbf{x})$ considering spatial correlations. Prediction is in general achieved through kriging techniques (Matheron 1965).

In the following analysis, ordinary kriging is used to predict the value of $S(\mathbf{x})$ for every location $\mathbf{x}$ in the area, from a linear combination of the observed values $\{Z(\mathbf{x}_i), i = 1, \ldots, g\}$. Note that as $Z(\mathbf{x})$ and $S(\mathbf{x})$ differ only through i.i.d. observation error, we can model the spatial covariance of $Z(\mathbf{x})$ directly instead of that of $S(\mathbf{x})$. Ordinary kriging requires the stationarity of the first differences of $Z(\mathbf{x})$, also called the intrinsic hypothesis by Matheron (Cressie 1986):

$$E(Z(\mathbf{x} + \mathbf{h}) - Z(\mathbf{x})) = 0$$
$$V(Z(\mathbf{x} + \mathbf{h}) - Z(\mathbf{x})) = 2\gamma(\mathbf{h}) \quad (2)$$

where $\mathbf{h}$ is a vector of length $h = |\mathbf{h}|$. The first equation means that $Z(\mathbf{x})$ (and thus $S(\mathbf{x})$) has same expected value over the whole area regardless of location; in other words, $\mu(\mathbf{x}) = \mu$. This average value is assumed to be unknown in ordinary kriging. Under the second condition, the variance of the difference is a function of $\mathbf{h}$ only. If we assume in addition that the process is second-order stationary, i.e.,

$$\mathrm{Cov}(Z(\mathbf{x} + \mathbf{h}), Z(\mathbf{x})) = C(\mathbf{h}),$$

the covariance between two points is only a function of their relative position, the semivariogram can then be expressed in terms of the spatial covariance as

$$\gamma(\mathbf{h}) = \sigma^2 - C(\mathbf{h}) \quad (3)$$

where the sill σ^2 is the variance of $Z(\mathbf{x})$ in the model (Journel and Huijbregts 1978, p. 163). The semivariogram (or simply

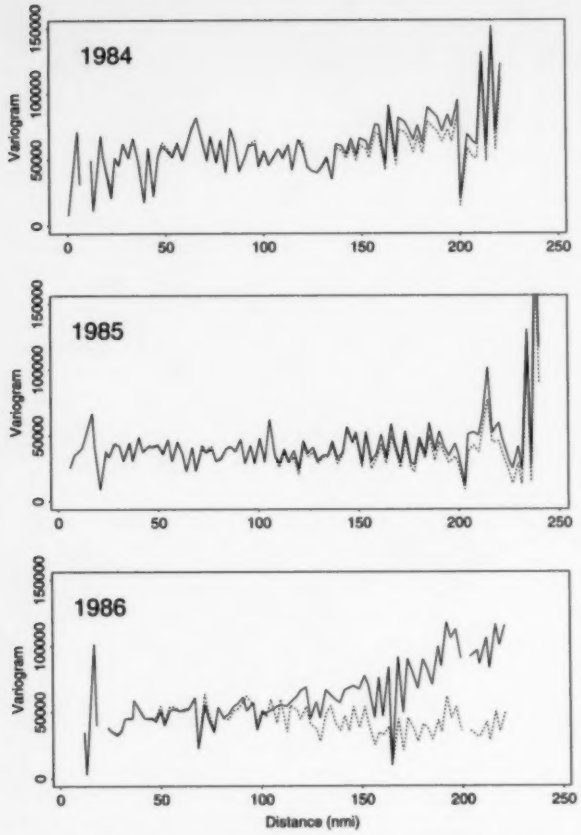

FIG. 5. Empirical variograms based on catch per set information for 1984, 1985, and 1986. The solid line represents the variogram estimated from the raw data; the broken line is the variogram obtained with data detrended from the east–west effect. There are 100 equally spaced bins.

variogram) $\gamma(\mathbf{h})$ thus characterizes the spatial correlation structure of $Z(\mathbf{x})$.

In general, $\gamma(\mathbf{h})$ is not known and has to be estimated empirically from the data. In this respect, the survey design used by IPHC has limitations in that it is too unbalanced, with stations very close together along north–south transects and transects too far apart from each other. As a consequence, information for small distances was only available in a north–south direction, and it was not possible to compute $\gamma(\mathbf{h})$ for different directions. A nondirectional variogram was thus computed by pooling pairs of points in all directions using Matheron's (1965) classical estimator:

$$(4) \qquad 2\widehat{\gamma(h)} = \frac{1}{N(h)} \sum_{i=1}^{N(h)} (Z(\mathbf{x}_i + \mathbf{h}_i) - Z(\mathbf{x}_i))^2$$

where $N(h)$ is the number of distinct pairs of points which are h nautical miles (nmi) apart and $\mathbf{h}_i$ is the vector of length h that connects the ith pair of points. The use of a nondirectional variogram implies the assumption that the process is isotropic. In this case, the design makes it extremely difficult to test this assumption. A variogram was computed in the northeast–southwest direction for distances up to 100 nmi, and it showed the same structure as the nondirectional one. However, these results (not reported here) are far too limited to really support the isotropy assumption. In order to compute $\widehat{\gamma(h)}$, the distance between all pairs of surveyed stations was discretized and the pairs were assigned to distance bins. Pairs of points in each bin were actually a distance $h \pm$ half the width of the bin apart. The number of bins was 100, each corresponding to 2.5 nmi, from 0 to 250 nmi.

For each year, results showed higher variogram values at large distances, which indicates a nonstationary problem (Fig. 5). Some of these values relied on very few pairs of stations (e.g., in 1984 and 1985) and should thus be downweighted when modelling the variogram. Actually, most of them pertained to the east–west effect quoted in the previous section, particularly in 1986. Such an effect was removed by subtracting from each local CPUE the average CPUE in the corresponding area (east or west). A variogram could then be estimated from the residuals. The resulting variograms for 1984–86 appeared to be stationary, except for some influential points in 1984 and 1985 (Fig. 5). They stabilized around 20 nmi for 1984 and 1985, and 50 nmi for 1986.

In general, such empirical variogram estimates are not conditionally negative-definite, which may lead to negative mean-squared errors of prediction (Cressie 1991, chap. 2). It is hence necessary to fit a valid variogram model to the estimates. Among the valid models, an exponential model (Journel and Huijbregts 1978) was chosen in our case for two reasons. First, in such a model, the decay of spatial correlation with distance is continuous, unlike a spherical model, for instance. This seems more relevant for a process like the abundance of benthic species. Second, this model gave the best fits. The exponential model is defined by

$$(5) \qquad \gamma(h) = \sigma^2 - (\sigma^2 - c_0)\exp\left\{\frac{-3h}{r}\right\}$$

where the practical range r (Journel and Huijbregts 1978, p. 164) is the distance beyond which there is virtually no correlation left between the abundances at two points, the sill σ^2 is identical to the variance of $Z(\mathbf{x})$ under a second-order stationarity assumption (see equation (3)), and the nugget effect c_0 corresponds to unobserved small-scale variance and observation error.

The exponential model was fitted to the variogram estimates of each of the three years using an approximate weighted-least-squares procedure (Cressie 1991, chap. 2) by minimizing

$$(6) \qquad \sum_{i=1}^{H} N(h_i)\left(\frac{\widehat{\gamma(h_i)}}{\gamma(h_i)} - 1\right)^2$$

where H is the number of bins into which the distances were discretized. Each squared residual was thus weighted according to $N(h_i)$, the number of pairs of points used to compute $\widehat{\gamma(h_i)}$, and to the inverse of $\gamma(h_i)^2$. This downweights nonrepresentative and generally outlying points and gives more weight to the points near the origin, which is the crucial part in determining the variogram parameters. Cross-validation could also be used to estimate the parameters, but it was computationally prohibitive in our case (see Simard et al. (1992) for an application). In order to obtain the fit, an alternative parameterization of the exponential model was used:

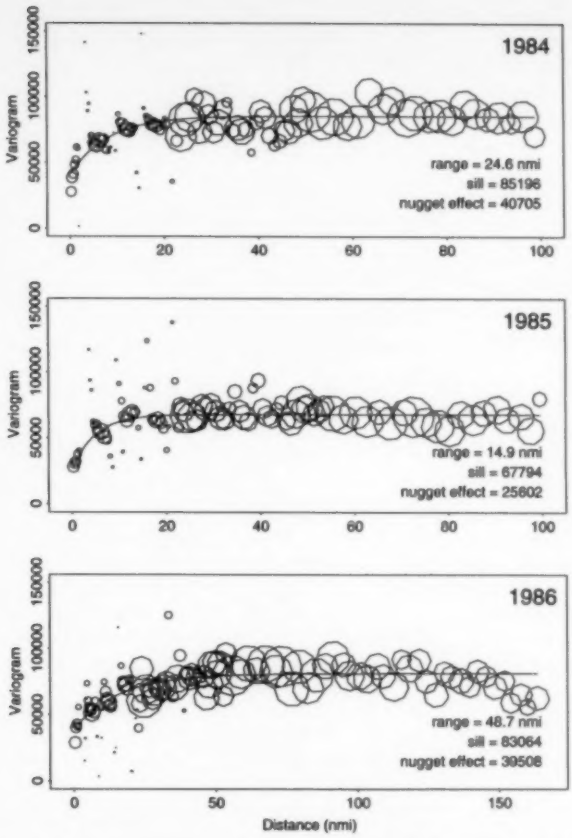

FIG. 6. Variograms based on catch per skate information for 1984, 1985, and 1986. The circles correspond to the empirical variogram; the solid line is the modelled variogram. Circle radii are proportional to the number of pairs of points from which each variogram estimate was computed. There are approximately 100 bins (depending on the year) emphasizing small distances. Note the difference in the x-axis for 1986, due to a larger range.

$$p_1 = \exp\left(-\frac{3}{r}\right)$$
$$p_2 = \sigma^2 - c_0$$
$$p_3 = c_0.$$

Resulting estimates had a reduced bias and were more stable numerically. Unfortunately, even in these conditions, it was impossible to estimate correctly the nugget effect and to a lesser extent the range from the variograms in Fig. 5. This is because the variogram estimates were not very informative for small distances. For instance, in 1985 the range was around 15–20 nmi; consequently, only a few points of the variogram contained information about both range and nugget effect. Given the relatively low value of the range, it was clear that a smaller scale of observation would be helpful in identifying these parameters. Besides, the nugget effect includes by definition variation at scales smaller than the minimum distance between observations (Journel and Huijbregts 1978, p. 152), i.e., 6 nmi in this case. Changes in the patterns of variations at small distances may possibly be evidenced from observations at a smaller scale. In order to model the variogram correctly, it was thus necessary to provide additional information regarding small-scale variation.

TABLE 2. Estimated parameters for the per-skate variograms (standard deviation of each estimate given in parentheses).

	p_1	p_2	p_3
1984	0.8864 (0.02)	44 491 (4400)	40 705 (4500)
1985	0.8172 (0.04)	42 192 (3880)	25 602 (3870)
1986	0.9412 (0.01)	43 556 (5906)	39 508 (6090)

Change of Support

In this case, small-scale information was available as CPUE recorded for each skate within each set (see description of survey data). CPUE per skate was thus used to improve the estimation of the parameters of the variograms. The locations of the skate centers were computed from the location of the set centers and the skate lengths. The minimum distance between two observations became 0.3 nmi approximately, compared with 6 nmi with the per-set CPUE. In order for the discretization of distances to reflect the empirical distribution of distances over all pairs of points, bins were constructed so that the average distance in the first (respectively second) bin corresponded to the distance between adjacent skates (respectively between skates separated by one skate), and so on. Finally, as the information about the spatial correlation was contained in the first part of the variogram (due to relatively small ranges), variograms were estimated only for distances up to 100 nmi for 1984 and 1985 (165 nmi for 1986, which variogram exhibits a larger range). Varying the actual truncation distance by ±40 nmi did not affect the results of the estimation. As with the per-set data, the east–west effect was removed before estimating the variograms. The estimates obtained were satisfactory in terms of both precision and numerical stability (Fig. 6; Table 2). The weight of each point in the fitting criterion helped to discriminate the representative points, particularly near the origin. A relatively weak spatial correlation was found in 1984 and 1985 whereas a larger range was estimated for 1986 (Fig. 6).

The per-skate variograms are by definition more informative at small distances than the per-set variograms (Fig. 5 and 6). Thus, by resorting to per-skate data, one could have found additional small-scale structures, i.e., structures with a range smaller than the smallest distance between per-set observations (6 nmi). Such structures would not be apparent at the larger observation scale. For all three years, results showed instead that the variance decreased rather smoothly towards the origin and there were no small-scale structures in the range 0.3–6 nmi. The estimates of nugget effect correspond to the variance that remains close to the origin. We will assume that this variance was all due to observation error, i.e., the difference between the observed CPUE at location $\mathbf{x}$, $Z(\mathbf{x})$, and the actual fish density at that location, $S(\mathbf{x})$. This assumption implies that the true process is L_2-continuous (i.e., the variogram of $S(\mathbf{x})$ is continuous with zero nugget effect), but that the observations are noisy so that two arbitrarily close observations may still differ. Year-to-year differences in the nugget effect estimates are thus not surprising, since survey conditions may vary from year to year. A more common, although usually implicit, assumption made in kriging applications is that there is no observation

TABLE 3. Regularized parameters for the per-set variograms. The spatially structured component corresponds to the ratio $(\hat{\sigma}^2 - c_0)/\hat{\sigma}^2$.

	Range (nim)	Sill	Nugget effect	Spatially structured component (%)
1984	24.6	46 264	5088	89
1985	14.9	41 969	4267	90
1986	48.7	46 707	6585	86

error and c_0 reflects unobserved microscale variability, i.e., small "nuggets" in the process (see Cressie 1991, p. 59). In reality, both observation error and microscale variation probably contribute to c_0 and it is not possible to estimate from the data how c_0 should be partitioned between the two. As we shall see below, the estimates of nugget effect for the per-set data are low relative to the sills and our assumption is thus of relatively minor consequence upon the results.

The parameter estimates obtained from the fitting could not be used as such for kriging the per-set CPUE because they pertain to the variograms of the raw catch per skate. Recall that the per-set CPUE corresponds to the *average* catch per skate within a set of six (or eight) skates. The per-skate variable had to be "regularized" (Journel and Huijbregts 1978, p. 77 and seq.), i.e., averaged over a set, to yield the per-set variable. This change of support (in the geostatistical terminology) affects spatial correlation in the same way that averaging smoothes variation and reduces variance. For distances larger than the size of support (i.e., the set length, approximately 2 nmi), the regularized per-set variogram can be approximated as a simple function of the per-skate variogram:

$$(7) \qquad \gamma_{\text{set}}(h) \simeq \gamma_{\text{skate}}(h) - \bar{\gamma}_{\text{within-set}}$$

where $\bar{\gamma}_{\text{within-set}}$ represents the mean value of $\gamma_{\text{skate}}(h)$ over all possible pairs of skates within a set. This approximation implies that if $\gamma_{\text{skate}}(h)$ is exponential, then $\gamma_{\text{set}}(h)$ is exponential too with an unchanged range, although the range theoretically increases as a consequence of smoothing by averaging.

Because the per-set CPUE is the mean of the catches per skate in that set, the regularized sill may be derived easily, yielding

$$\sigma^2_{\text{set}} = \sigma^2_{\text{skate}} - \frac{1}{n^2}\sum_{i \neq j} \gamma_{\text{skate}}(i,j)$$

where n is the number of skates per set and i,j index the skates in a given set. This equation can also be deduced by replacing equation (5) into (7) and identifying all terms that do not involve h.

Since it is assumed that the nugget effect of the per-skate variogram only represents observation error, i.e., no small-scale structure, and since the per-skate empirical variograms did not show any additional structure, the regularized nugget effect (i.e., the nugget effect for the per-set CPUE) can also be obtained as the variance of the average of the per-skate errors, leading to

$$(8) \qquad c_0^{\text{set}} = \frac{c_0^{\text{skate}}}{n}.$$

A less intuitive although more rigorous derivation results from noticing that the condition $\sigma^2_{\text{set}} - c_0^{\text{set}} = \sigma^2_{\text{skate}} - c_0^{\text{skate}}$ is needed for equation (7) to hold. Thus,

$$c_0^{\text{set}} = c_0^{\text{skate}} - \frac{1}{n^2}\sum_{i \neq j} \gamma_{\text{skate}}(i,j),$$

which approaches (8) when distances between skates within a set tend to zero, and

$$\sum_{i \neq j} \gamma_{\text{skate}}(i,j) \rightarrow (n^2 - n) c_0^{\text{skate}}.$$

Notice that, like variances, both sill and nugget effect decrease when averaging.

Regularized parameter estimates indicate a relatively weak spatial correlation (in terms of range) for 1984 and 1985, but a much stronger one for 1986 (Table 3). Nugget effects were rather low for all three years, accounting for only 10–15% of the a priori variance.

Spatial Distribution of Halibut Abundance

Spatial Prediction Using Kriging

Halibut abundance at each location was predicted as a weighted average of the observations, with weights being a function of the spatial covariance described by the modelled variograms. Recall that the per-set data were kriged. Alternatively, we could have kriged the per-skate data directly along with the corresponding variograms, but this is computationally more intensive. Once detrended from the east–west effect, the process was considered stationary. As the expected value of the process remained a priori unknown, halibut abundance at each location $\mathbf{x}_0$ was predicted using ordinary kriging (Matheron 1965) as

$$\hat{S}(\mathbf{x}_0) = \sum_{i=1}^{g} \lambda_i Z(\mathbf{x}_i)$$

where the vector of weights λ minimizes the prediction error $E(S(\mathbf{x}_0) - \hat{S}(\mathbf{x}_0))^2$ subject to the constraint that $\Sigma_i \lambda_i = 1$ so that $\hat{S}(\mathbf{x}_0)$ is unbiased (see Cressie 1991, chap. 3, for a derivation of kriging equations). It is important to remark that kriging was used in this analysis to predict the actual abundance $S(\mathbf{x})$, rather than $Z(\mathbf{x})$ which corresponds to the process distorted by white noise. This requires an estimate of the variance of the observation error, which we assumed was equal to the nugget effect c_0. A consequence of allowing for observation error is that kriging predictions do not honor the data; they smooth them instead, as is sensible when the data are known to be noisy. This is contrary to most kriging applications: standard kriging equations assume zero observation error, thus leading to an exact interpolation, i.e., they predict $Z(\mathbf{x})$ instead of $S(\mathbf{x})$. As explained by Cressie (1991, p. 128–129), this distinction is not important for the kriging maps (except at the actual data locations), but it is relevant for the computation of the prediction error (also known as kriging variance). This latter is now equal to

$$(9) \qquad V_0^* = E(S(\mathbf{x}_0) - \hat{S}(\mathbf{x}_0))^2$$

$$= 2\sum_{i=1}^{g} \lambda_i \gamma(\mathbf{x}_0 - \mathbf{x}_i) - \sum_{i=1}^{g}\sum_{j=1}^{g} \lambda_i \lambda_j \gamma(\mathbf{x}_i - \mathbf{x}_j) - \sigma_v^2$$

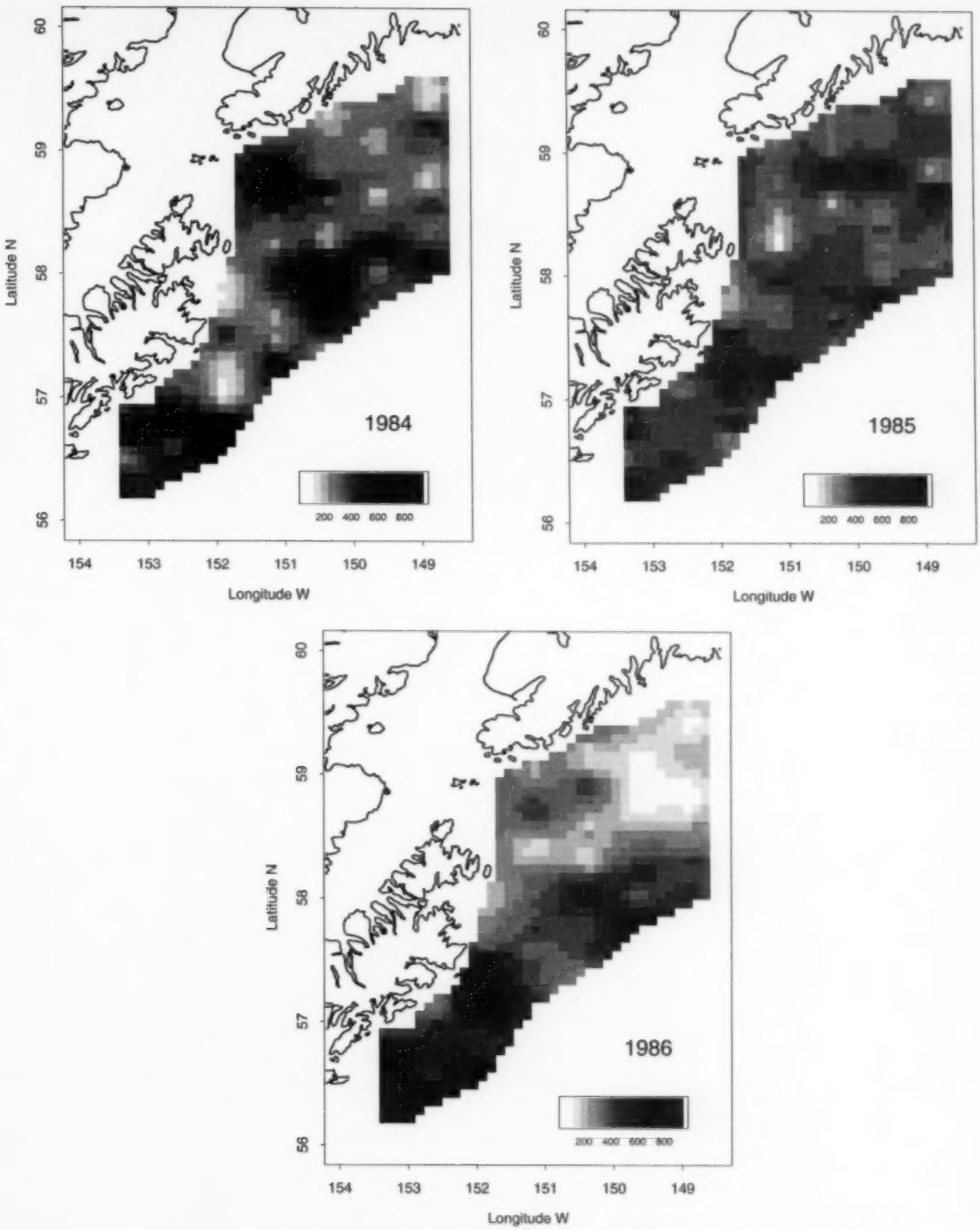

FIG. 7. Kriging maps for 1984, 1985, and 1986. Grey levels are the same for the three years.

where σ_ν^2 is the variance of the observation error which we assumed was equal to c_0.

Kriging was achieved over a grid of squared cells of approximate length 4 nmi. The southern border of the kriged area was defined as the limit of the continental shelf beyond which halibut abundance is believed to be negligible during the feeding season. Eastern and western borders approximately correspond to the limits of the surveyed area. Global

TABLE 4. Global estimates of relative abundance (net catch per skate) obtained by kriging the CPUE data.

Year	No. of stations	Mean	Global variance
1984	104	370.8	338
1985	97	374.6	403
1986	99	343.7	256

mean abundances and global variances, i.e., associated prediction errors, were computed by combining local estimates over the whole area (Journel and Huijbregts 1978, p. 323):

$$S^*_{\text{global}} = \frac{1}{G}\sum_{i=1}^{G} \hat{S}(\mathbf{x}_i)$$

(10)

$$V^*_{\text{global}} = \frac{1}{G^2}\left(\sum_{i=1}^{G} V_i^* + \sum_{i\neq j} \text{Cov}^*_{ij}\right)$$

where G is the number of kriged cells, $\hat{S}(\mathbf{x}_i)$ and V_i^* are, respectively, the kriging estimate and the associated kriging variance at cell i, and Cov^*_{ij} is the error covariance between cell i and cell j:

$$\text{Cov}^*_{ij} = E[(S(\mathbf{x}_i) - \hat{S}(\mathbf{x}_i))(S(\mathbf{x}_j) - \hat{S}(\mathbf{x}_j))],$$

which can be expressed in terms of the kriging weights and variogram function, like in equation (9).

Notice from equation (9) that the effect of assuming that $\sigma^2_\nu = c_0$ as opposed to setting $\sigma^2_\nu = 0$, i.e., assuming that the nugget effect is only observation error versus only microscale variation, is to reduce the estimated prediction error V_0^*. The closer the grid point $\mathbf{x}_0$ is to actual observations, the larger the relative error reduction at that point. On the other hand, for points far apart from observation locations, and thus with a larger prediction error, the relative error reduction decreases and tends to the ratio between σ^2_ν and the sill.

Consequently, the prediction error for the average abundance over the whole area (global variance in equation (10)) is reduced by σ^2_ν/G. In our case, the ratio σ^2_ν/sill was 0.11, 0.10, and 0.14 for 1984, 1985, and 1986, respectively. This amounted to a small relative reduction in the global variance (1.8, 1.2, and 3%, respectively, for the three years) with respect to the error estimated by assuming $\sigma^2_\nu = 0$.

Results

The abundance maps predicted by kriging (Fig. 7) show a relative persistence of the spatial distribution during the years surveyed. At large scales, this is in relation to the stability of the east–west effect and could be detected directly from the raw data. At smaller scales, the variogram parameters indicated some stability in the distribution, in that the spatially structured component of the abundance was remarkably constant over the three years (Table 3, last column). Maps for 1984 and 1986 exhibit relatively similar locations for most patches of high abundance, e.g., around 57°30′–58° latitude and 149°–150° longitude, or 56°30′ and 152°. Some areas of low abundance have similar locations too, e.g., at 59° and 149°30′, which contrasts with 1985. In general, high abundance areas in 1985 did not coincide with those in other years.

These features of the spatial distribution may partly be explained by both bottom topography (Fig. 4) and the timing of the surveys. In 1984 and 1986, high abundances were found in flat areas like Albatross Bank and Portlock Bank, and even closer to the shore at longitude 151°, but also in gullies, e.g., Albatross Gully and Chiniak Gully. In 1985, highs were systematically associated to gullies, particularly Seward Gully and also Albatross and Chiniak. Recall that this survey took place in Spring (May), while the 1984 and 1986 surveys were conducted in summer (July–August). The 1985 pattern seems to indicate that migration from the deep winter grounds was not yet completed at the time of the survey. Apparently, halibut migrate essentially via the gullies (and not continuously along the continental slope), before scattering in some preferred areas, mainly flats. Of course, this scenario needs further confirmation, but the maps obtained provide some clues. Topography could thus explain some persistent patterns observed in the maps, which would not be entirely surprising for this benthic species. Regarding smaller scale, results for the three years showed no small-scale structures and a rather low nugget effect. There were, however, year-to-year differences, like the higher variogram range observed in 1986. The global abundance estimate for 1986 was significantly lower than in 1984 and 1985 (Table 4), and the east–west effect was much stronger (Table 1).

Testing a New Sampling Design

Representing the mean squared prediction error, i.e., the kriging variance, stresses the importance of the survey design for the precision of the prediction (Fig. 8). The asymmetry of the grid generated a relatively poor prediction between transects. This was all the more striking as the range decreased (e.g., see 1984 and 1985 compared with 1986). Hence, the analysis provided some guidelines for improving the survey design.

IPHC has resumed annual surveys of the area off Kodiak using a new, closer-to-regular sampling design (Fig. 9, solid symbols). The distance between transects is now 12 nmi, and distances between stations along a transect line range from 12 to 18 nmi. This new design departs from a perfectly regular grid, which would be optimal from a geostatistical standpoint (Yfantis et al. 1987) because of logistic constraints. The most critical one is that the number of stations that can be completed in a fishing day decreases with the distance between stations, so covering a more regular grid would require prolonging further the duration of the survey, which is not feasible. In the present grid, stations are arranged in clusters of three (triangles) that can be fished in a day, and a fourth station is placed at random within each triangle (not represented in Fig. 9).

For the purpose of evaluating the efficiency of the new sampling design, as the fixed grid has fewer stations than the old one, the fourth station was systematically positioned at the center of each triangle. This slight difference with respect to the design actually implemented is of minor consequence upon the following test. The performance of this design was evaluated here based on the estimated spatial covariances in 1985 and 1986. Recall that 1985 exhibited a rather low range in contrast with 1986, so that it is possible to compare the effect of different variogram parameters upon the performance of a design.

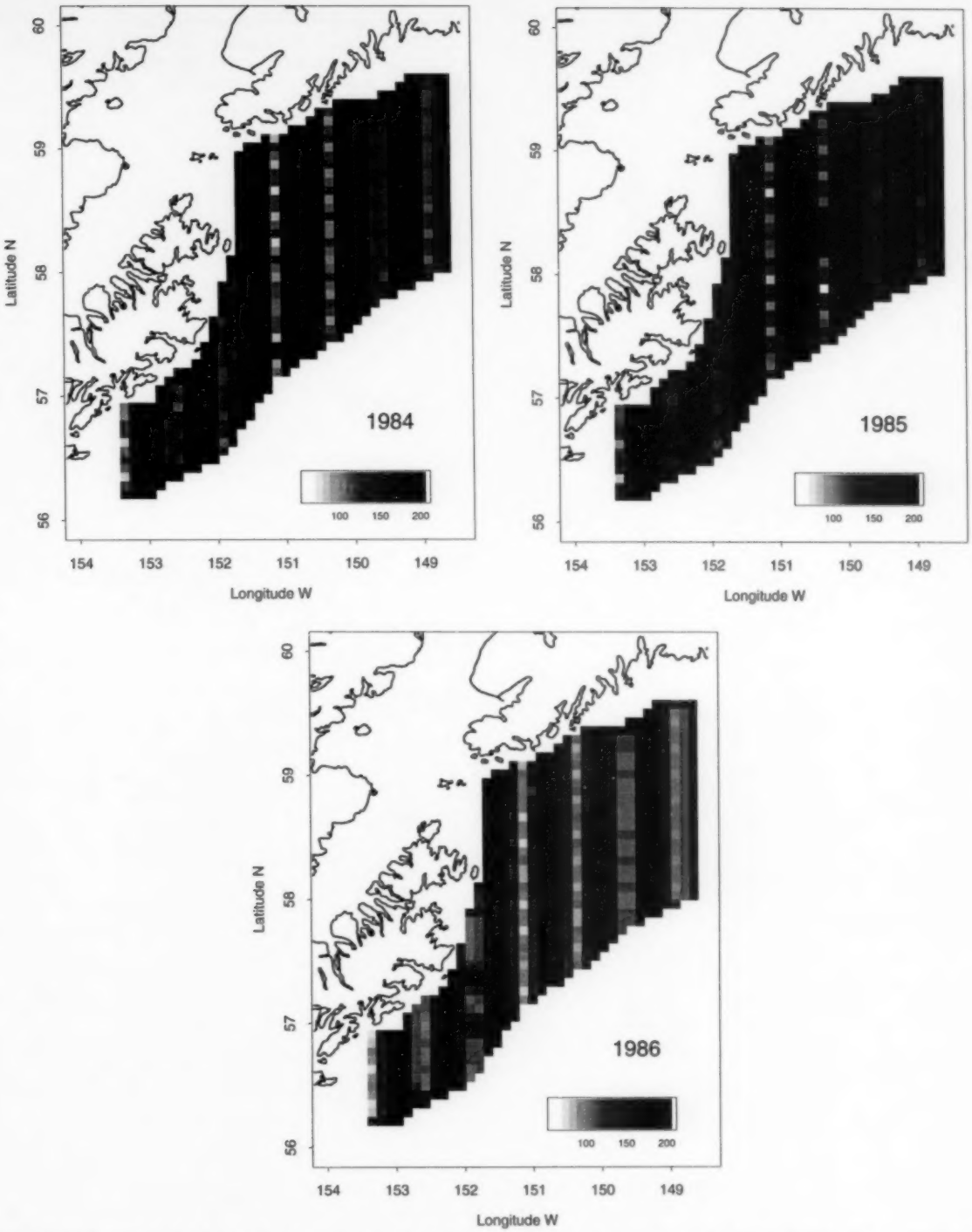

FIG. 8. Kriging standard deviations (i.e., square root of the local kriging variance) for 1984, 1985, and 1986. The spatial pattern reflects the sampling design. Grey levels are the same for the three years.

Kriging variances and covariances only depend on both variogram and sampling design; they depend on the data only through the variogram. Therefore, given a variogram and the locations of the sampling stations, it is possible to solve the kriging equations to obtain the weights λ and therefore the local kriging variances V^* and covariances Cov^*. These

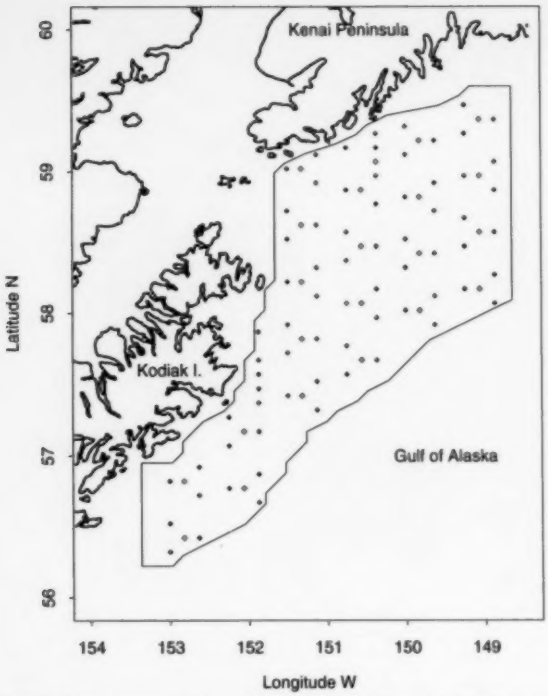

FIG. 9. New survey design: solid symbols show the fixed grid of stations arranged in clusters of three; empty symbols correspond to additional stations which are placed at random within each triangle in the real design (they were here positioned exactly at the center for the purpose of evaluating kriging variances). The solid line delineates the area over which all maps and global indices are computed.

allow, then, computation of a prediction error for the estimated average CPUE over the area covered by the survey (i.e., a global variance as defined earlier). The kriging variances obtained using the new design differed strikingly from the previous ones. First, they were much more homogeneous over the whole area (Fig. 10). Second, the variance of the global predictor was decreased by approximately 20% (Table 5) with only 93 stations (compared with 97 and 99 for 1985 and 1986, respectively). The new design resulted in slightly smaller variances than the old one, even when the stations at the center of the triangles were excluded (leaving only 71 stations). For any given design, absolute variances differed markedly between the two years in relation to the different variograms (Table 5; Fig. 8 and 10). However, the relative gains in variance induced by changing the design were almost identical for both years.

Discussion

The application of variographic analysis and kriging to longline CPUE gives encouraging results, even though the data collection was not designed for this purpose. The maps obtained along with the variograms and the global estimates provide insight into the spatial distribution of halibut, even if the proposed explanations are rather conjectural given the few years considered. The results show the potential usefulness of modelling the spatial correlation observed in the data for estimating abundance maps.

In addition, this application illustrates two methodological points that seem important to discuss: (i) the necessity of small-scale information for the variographic analysis and (ii) the advantages of using a sampling design more adapted to analyses based on spatial correlations.

Uncertainty about Small-Scale Spatial Structure

The existence of detailed information, i.e., the catch per skate, was essential in the analysis of the spatial structure through the variogram, mainly because of the small structures found in 1984 and 1985. Two scales of observation brought different insight into the spatial distribution of the process of interest, the smallest one allowing refinement of the modelling of the variogram via a better estimation of the nugget effect and the range. This was made possible by the gear used; it is indeed relatively easy with a longline to record the catch for short segments within a set. One could argue that trawl surveys may not be suitable for this change of support. Note incidentally that a longline of 2 nmi, i.e., approximately the length of the sets in the survey data analyzed here, has same length as a haul of 30 min at a speed of 4 kn.

In addition to a background grid of regularly spaced stations, one could devote some time during the survey to an experiment of closer stations in a limited area. Such an experiment is under study at the French Institute for the Exploitation of the Sea (IFREMER). This may not always be simple depending on the gear used. Standardization problems may for instance arise if placing the stations close together results in interference between adjacent pieces of gear (as with pots), or if it requires shortening tows. This is because the efficiency of trawl or dredge gear may vary with the duration of the tow, so that hauls of different lengths may not be comparable. Alternatively, it is possible to allocate a number of additional stations in different places so as to generate closer pairs of points (as for instance in the design evaluated in the previous section). Hence, there are reasonable design changes that may help to cope with the small-scale uncertainty about the spatial structure.

Design Limitations: Possible Improvements

Even though the nature of the data collected was a posteriori found appropriate to analyze the spatial structure of CPUE, there remains a problem tied to the sampling design, namely its asymmetry. North–south transects 12 nmi apart were initially defined by IPHC because (i) depth and distance to the shore were thought to be determinant of the distribution of halibut abundance (Hoag et al. 1980) and (ii) it is a classical approach in fisheries. Later, the suppression for cost reasons of every other transect resulted in this markedly unbalanced design (Fig. 1). A consequence of this limitation on the variographic analysis is that the estimation of the variogram at small distances is mostly based on within-transect pairs of points. The assumption of an isotropic spatial distribution is thus required for the estimated variogram to be applicable to all directions in the space. Such an assumption may not be totally unrealistic because the region of interest is included in the continental shelf with relatively homogeneous environmental conditions. But the main problem lies in the impossibility of verifying this assumption from the available data. Another limitation due to the unbalance of the design is the relatively poor precision of kriging predictions between the transects, especially for years with smaller spatial structures. A regu-

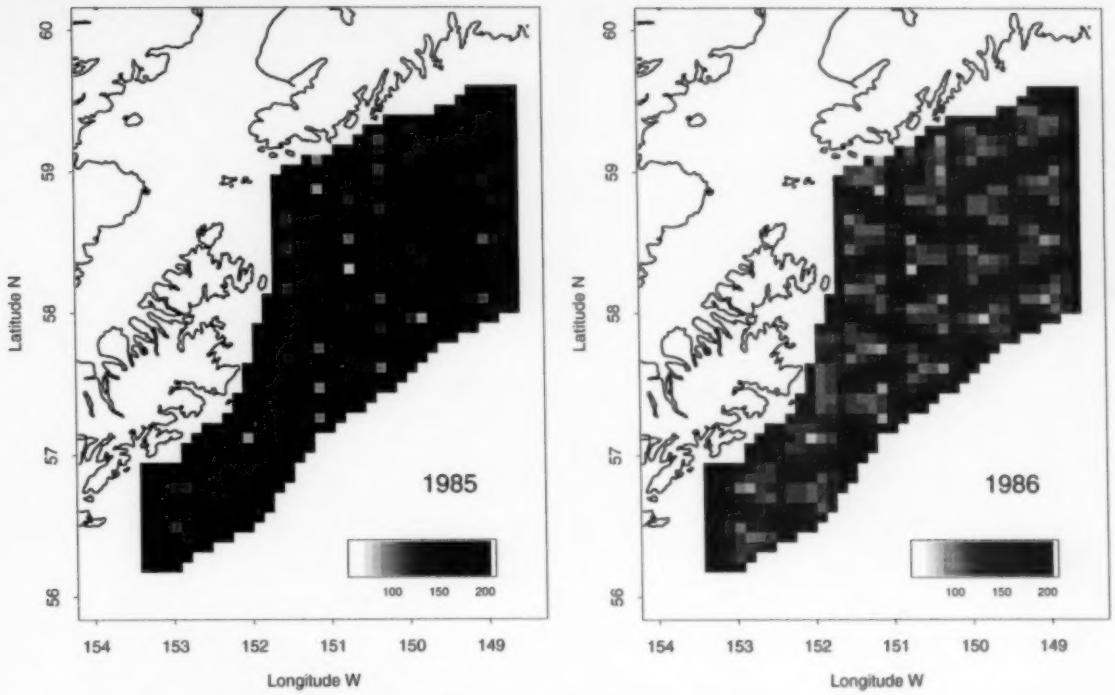

FIG. 10. Kriging standard deviations obtained for 1985 and 1986 using the new survey design and previous variograms.

TABLE 5. Global variances obtained for different sampling designs. The variances are computed over the same area as in the previous maps.

Year	Design	No. of stations	Global variance
1985	Old	97	403
1985	Triangles + centers	93	326
1985	Triangles	71	396
1986	Old	99	256
1986	Triangles + centers	93	213
1986	Triangles	71	247

lar design with equally spaced stations is more desirable for the application of geostatistical techniques, as exemplified by our results.

Finally, this analysis entails several perspectives. First, it is necessary to investigate more closely the habitat preference of the species in relation to topography and bottom type. In this regard, the data from future surveys will provide finer information about depth. In addition, the new sampling design should help to solve some of the problems encountered here and will permit the evaluation of possible anisotropies in the distribution of Pacific halibut. Size- and sex-dependent spatial distributions could also be considered. A further avenue for future research lies in the analysis of commercial CPUE in conjunction with the maps obtained from the surveys.

Acknowledgements

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Optimum Exercise Conditioning Velocity for Growth, Muscular Development, and Swimming Performance in Young-of-the-Year Striped Bass (*Morone saxatilis*)

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Young, P.S., and J.J. Cech, Jr. 1994. Optimum exercise conditioning velocity for growth, muscular development, and swimming performance in young-of-the-year striped bass (*Morone saxatilis*). Can. J. Fish. Aquat. Sci. 51: 1519–1527.

Young-of-the-year striped bass (*Morone saxatilis*) were exercise conditioned for 60 d at four different water velocities: <0.02 (control), 0.5–1.2 (slow), 1.5–2.4 (moderate), and 2.4–3.6 body lengths·s^{-1} (fast). Sampling for growth (body weight change), muscular development (red and white muscle cross-sectional areas), and swimming performance (critical swimming velocity) was done at 50 and 60 d of the exercise conditioning period and at 14, 28, 42, and 56 d post-conditioning. Optimum conditioning velocities for growth and swimming performance were the moderate and fast velocities, respectively, with both effects persisting 56 d post-conditioning. Fish exercised at the moderate and fast velocities showed significant increases in red muscle areas (at 0 and 14 d post-conditioning, respectively) and in white muscle areas (at 0–28 and 14–28 d post-conditioning, respectively). Exercise-conditioned fish at any velocity showed a significant increase in the red to white muscle ratio at the end of 60 d of conditioning. Our data suggest that exercise-conditioned young-of-the-year striped bass would have size and swimming advantages consistent with higher survival than unexercised fish when stocked in natural ecosystems.

Des jeunes de l'année du bar d'Amérique (*Morone saxatilis*) ont été conditionnés à l'exercice pendant 60 jours à quatre vitesses de déplacement dans l'eau différentes : <0,02 (témoin), 0,5 à 1,2 (lente), 1,5 à 2,4 (modérée) et 2,4 à 3,6 longueur de corps·s^{-1} (rapide). L'échantillonnage pour la mesure de la croissance (changement du poids corporel), du développement musculaire (surface de section transversale des muscles rouges et blancs) et de la performance natatoire (vitesse natatoire critique) a été effectué aux jours 50 et 60 de la période de conditionnement à l'exercice et aux jours 14, 28, 42 et 56 après le conditionnement. Les vitesses de conditionnement optimales pour la croissance et la performance natatoire sont respectivement la vitesse modérée et rapide, les deux effets persistant 56 jours après le conditionnement. Chez les poissons conditionnés à l'exercice aux vitesses modérée et rapide, on observe des augmentations significatives de l'aire de surface des muscles rouges (à 0 et 14 jours respectivement après le conditionnement) et des muscles blancs (respectivement entre 0 et 28 jours et 14 et 28 jours après le conditionnement). Chez les poissons conditionnés à l'exercice, quelle que soit la vitesse, on a observé une augmentation significative du ratio muscles rouges à muscles blancs à la fin de la période de 60 jours de conditionnement. Nos données semblent indiquer que les jeunes de l'année du bar d'Amérique conditionnés à l'exercice pourraient posséder des avantages en matière de taille et de capacité natatoire propices à une survie plus élevée chez les poissons n'ayant pas fait d'exercice lors de leur empoissonnement dans les écosystèmes naturels.

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The population of striped bass (*Morone saxatilis*) in the Sacramento – San Joaquin estuary has declined rapidly since 1974 (Stevens et al. 1985) resulting in mitigative stocking of young-of-the-year (YOY) fish by the California Department of Fish and Game (CDFG). Stocked fish were raised in either large tanks (~3–6 m in diameter), raceways (~5 × 10 m^2), or ponds (~0.25–1.0 acre) (1 acre = 0.4 ha) and did not receive any exercise conditioning. The fact that the stocked fish were not conditioned is probably one of the reasons why the striped bass population continues to decline at an alarming rate in spite of the 11 million YOY striped bass stocked during 1981–91 (N. Villa, CDFG, personal communication). Our previous studies showed that exercise conditioning at 1.2–2.4 body lengths (bl)·s^{-1} for 60 d significantly improved growth, muscular development, and swimming performance (Young and Cech 1993a) and stress responses and recovery (Young and Cech 1993b) in cultured and wild YOY striped bass. In those studies, we also recommended exercise conditioning of striped bass used for mitigative stocking. However, an optimal exercise conditioning velocity was not determined.

Exercise conditioning in fish through forced continuous or intermittent swimming at different speeds and at different durations results in varied effects on growth, muscular devel-

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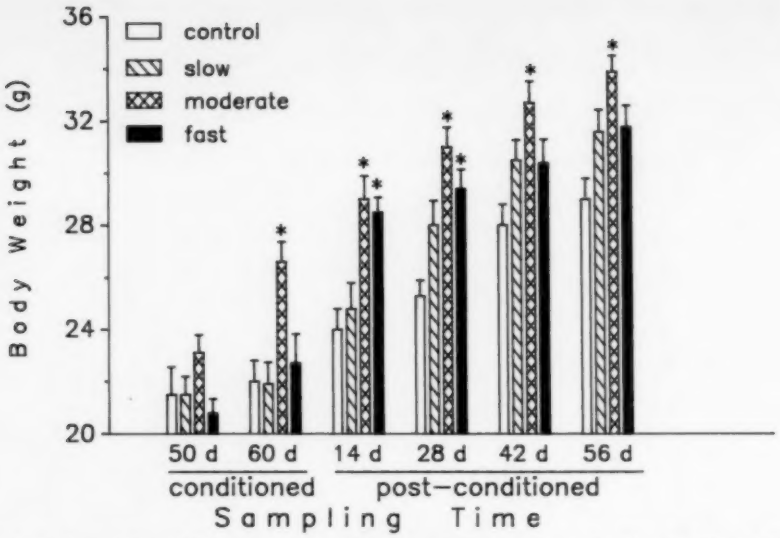

FIG. 1. Body weights of fish exercise conditioned at different flow velocities for 50 and 60 d and at 14, 28, 42, and 56 d post-conditioning (error bar = SEM; $n = 12$; *significantly greater than weight of control, $P < 0.05$).

opment, and swimming performance depending on fish species and the type of exercise regime (reviewed by Davison 1989). Growth rates are generally maximized in fish conditioned at intermediate swimming speeds, restrained at slower speeds or in standing water, but are reduced or minimal in fish conditioned at higher swimming speeds (e.g., Davison and Goldspink 1977; Greer Walker and Emerson 1978; Nahhas et al. 1982a; Besner and Smith 1983; East and Magnan 1987; Christiansen and Jobling 1990). Exercise conditioning has been shown to increase red muscles (Davison and Goldspink 1977; Greer Walker and Emerson 1978; Broughton et al. 1980; Johnston and Moon 1980a, 1980b; Nahhas et al. 1982a; Davie et al. 1986; Young and Cech 1993a), increase white muscles (Davison and Goldspink 1977, 1978; Greer Walker and Emerson 1978; Johnston and Moon 1980a; Totland et al. 1987; Young and Cech 1993a), or not affect muscle mass (Davie et al. 1986) depending on fish species and exercise conditioning regimes. Exercise conditioning generally improves swimming performance (e.g., Farlinger and Beamish 1978; Broughton et al. 1980; Nahhas et al. 1982b; Besner and Smith 1983; Leon 1986; Houlihan and Laurent 1987; Pearson et al. 1990; Young and Cech 1993a). However, differences in training regime result in variable responses. For example, increases in swimming performance of rainbow trout (*Oncorhynchus mykiss*) exercise conditioned for 28 d were found at speeds in excess of 2.5 $bl \cdot s^{-1}$ (Nahhas et al. 1982a). After being sprint-trained (i.e., chased vigorously around the tank with an electrified rod) for 30 s every other day for 9 wk, rainbow trout accelerated faster than untrained fish (Gamperl et al. 1991). Although sprint training may be important in predator avoidance, it did not improve rainbow trout critical swimming speed (Gamperl et al. 1991) and resulted in slower growth compared with untrained rainbow trout (Gamperl et al. 1988).

To our knowledge, no other study has determined optimal exercise conditioning velocities including persistence of the resulting effects. Persistence may be important for fish stocked into natural environments where larger size and better swimming ability would improve survival (Junge and Phinney 1963; Beamish 1978; Townsend and Calow 1981; Werner et al. 1983; Webb 1984, 1986; Folkvord and Hunter 1986; Margulies 1990). The main objective of our study was to ascertain the optimum exercise conditioning velocity for growth, muscular development, swimming performance and persistence of these effects in YOY striped bass.

Methods

YOY striped bass were obtained from the CDFG Central Valley Hatchery in Elk Grove. Fish were held in the laboratory for 2 wk in a 1 × 2.3 m fiberglass tank with a continuous flow of air-equilibrated well water and continuous aeration. After a 1-d fast, fish were individually weighed in a pretared beaker of water and randomly distributed among 12 plastic raceways. Each raceway was a black ABS plastic tank measuring 150 cm long, 30 cm wide, and 25 cm deep. A screen was placed cross-sectionally 30 cm from each end, and a PVC sheet longitudinally divided the inner section into two compartments. Each compartment was stocked with 15 fish. A Teel submersible pump with an attached flow diffuser was placed beyond the screen at each end of the raceway to generate the desired water velocity in the exercise-conditioned treatments. Each raceway had a water depth of 15 cm with a continuous flow of air-equilibrated well water at 2 $L \cdot min^{-1}$ creating a velocity of <1 $cm \cdot s^{-1}$.

The fish were subjected to four different water velocities: <1 (control), 2–6 (slow), 8–12 (moderate), and 12–18 $cm \cdot s^{-1}$ (fast). Teel submersible pumps of three different horsepowers (HP) were used to generate the different water velocities: 1/200 (slow), 1/35 (moderate), and 1/12 HP (fast). Each submersible pump had an attached rectangular (4 × 12 cm) flow diffuser to provide cross-sectionally uniform water velocity. Horizontally, however, the water

velocity was higher near the pump and lower farther from the pump (e.g,. in slow velocity, 6 $cm \cdot s^{-1}$ near the pump and 2 $cm \cdot s^{-1}$ farther from the pump). Control group tanks had no pumps. After 30 d, water velocity was increased to 8–12 (slow), 12–18 (moderate), and 18–34 $cm \cdot s^{-1}$ (fast) by either moving the pump nearer the fish compartment (moderate and fast velocities) or using a pump with more HP (slow velocity). The water velocity increases were necessary as fish grew to maintain velocities at 0.5–1.2 (slow), 1.5–2.4 (moderate), and 2.4–3.6 $bl \cdot s^{-1}$ (fast).

Water velocity was determined using a digital Marsh McBirney flowmeter. No "dead" spaces were found within the rearing compartments of the exercise-conditioned treatments. Temperature (YSI telethermometers, max:min thermometers), dissolved oxygen content (Nester DO meter), and ammonia content (Hach ammonia kit) of the water in each tank were monitored three times a week to ensure high water quality and minimal variability in these parameters in different tanks. Fish were kept in a 12 h light : 12 h dark photoperiod regime using an automatic timer.

Fish were gradually habituated to exercise conditioning for 5 d; thereafter, pumps were only stopped for feeding and tank maintenance. At the start of the conditioning, pumps were turned on only between 08:00 and 17:00 for 5 d, but were left on (except during feeding and cleaning) throughout the rest of the conditioning period (60 d total). Fish were fed with Silver Cup No. 2 trout pellets (Murray Elevators, Idaho) at about 7% body weight four times daily for the first 2 wk, three times daily for the next 2 wk, and twice daily for the rest of the rearing period. Pumps were turned off during feeding (allotted time = 20 min) and cleaning (20 min) for a total of 40 min per feeding and cleaning. Fecal matter and extra food were removed before the pumps were turned back on.

Fish mortality in some tanks occurred between 35 and 75 d of the rearing period due to gas bubble disease. Diagnosis of fish was done by the Fish Pathology Unit of the School of Veterinary Medicine at the University of California, Davis. The increases in dissolved gas occurred only during some nighttime and weekend periods due to a water supply pump shutdown. Installation of additional air-equilibration columns on the water supply alleviated the problem.

At the end of the 60-d conditioning period, all submersible pumps were removed. Feeding, cleaning procedure, and monitoring of physicochemical factors remained the same.

Sampling started at days 50 and 60 of the exercise conditioning period and at 14, 28, 42 and 56 d post-conditioning. A period of 3–4 d was needed for testing 48 fish per sampling time. At each sampling time, two fish from each compartment were individually tested for swimming performance (12 fish total for each treatment), using a modified Brett-type swimming apparatus (Brett 1964) and a procedure slightly modified from that described in Young and Cech (1993a). Briefly, individual fish were placed in the swimming chamber and, after a 10-min rest, critical swimming velocity was measured by step increases of 10 $cm \cdot s^{-1}$ in water velocity at 5-min intervals until the fish was fatigued (following Beamish 1978). After several preliminary runs, a 5-min interval was chosen instead of 2 min (Young and Cech 1993a) to ensure that most of the fish were fatigued before the maximum velocity was reached. A fish was considered fatigued when it was "pinned" three times at the rear of the chamber. No correction factor for solid blocking effect was applied because the fish occupied <10% of the cross-sectional area of the swimming chamber (Bell and Terhune 1970). The critical swimming velocity (U_{crit}) was calculated using the formula (Brett 1964)

$$U_{crit} = U_i + (U_{ii} \cdot T_i \cdot T_{ii}^{-1})$$

where U_i = highest velocity maintained for the prescribed time period, U_{ii} = velocity increment, T_i = time elapsed at final velocity, and T_{ii} = prescribed swimming period.

Immediately after the swimming performance test, each fish was quickly overanesthetized with MS-222, weighed, an frozen for muscle area measurements. Before each measurement, fish were partially thawed and cross-sectioned at 50, 65, and 80% SL and slide photographs of the sections were taken. Slides were projected onto a digitizing pad for tracing, and red and white muscle areas were calculated using the Microplan II imaging program.

Analyses of variance (ANOVA) and Tukey's test were used to find significant differences in body weights, red and white muscle cross-sectional areas, and U_{crit} of fish among different treatments per sampling time. Kruskal–Wallis ANOVA was used to determine significant differences in red to white muscle ratios. All statistical analyses were conducted using SYSTAT software.

Results

Temperature, dissolved oxygen, and ammonia contents of the water and fish mortality did not vary among different raceways. Daily mean temperature of the raceways fluctuated from a minimum of 18.7°C (±0.13 SE) at night to a maximum of 22.6°C (±0.13) during the day with an overall mean temperature of 20.7°C (±0.21). Dissolved oxygen content remained high (7.9 ± 0.07 $mg \cdot L^{-1}$) and ammonia content remained low (0.22 ± 0.02 ppm) throughout the rearing period. Fish mortality rate due to gas bubble disease ranged from 0 to 3 fish per compartment (mean = 1.2) but was not significantly different among treatments.

Control fish were observed to be oriented randomly within the compartment, but exercise-conditioned fish showed positive rheotaxis even at the slow-velocity treatment. Fish at fast velocities also exhibited a more synchronized locomotion.

Fish seemed to require the full 60-d conditioning period to show differences in growth. Initial weights (3.60 ± 0.03 g) of fish were not statistically different among treatments, and after a 50-d conditioning period, body weights increased sixfold but were still not statistically different among treatments (Fig. 1). Body weights of slow-velocity fish did not differ significantly from those of the controls throughout the rearing period. Body weights of the moderate-velocity fish were significantly greater ($P < 0.04$) than those of the controls after 60 d of conditioning and remained significantly greater after 56 d ($P < 0.05$) post-conditioning. It is interesting to note that the fast-velocity fish showed a significantly greater ($P < 0.05$) mean body weight than the controls only after 14 d ($P < 0.05$) and 28 d ($P < 0.03$) post-conditioning.

Sixty days of conditioning also seemed to be required for significant differences in muscular development. After a 50-d conditioning period, mean red and white muscle cross-sectional areas of fish at 50, 65, and 85% SL did not differ significantly among different treatments (Fig. 2 and 3).

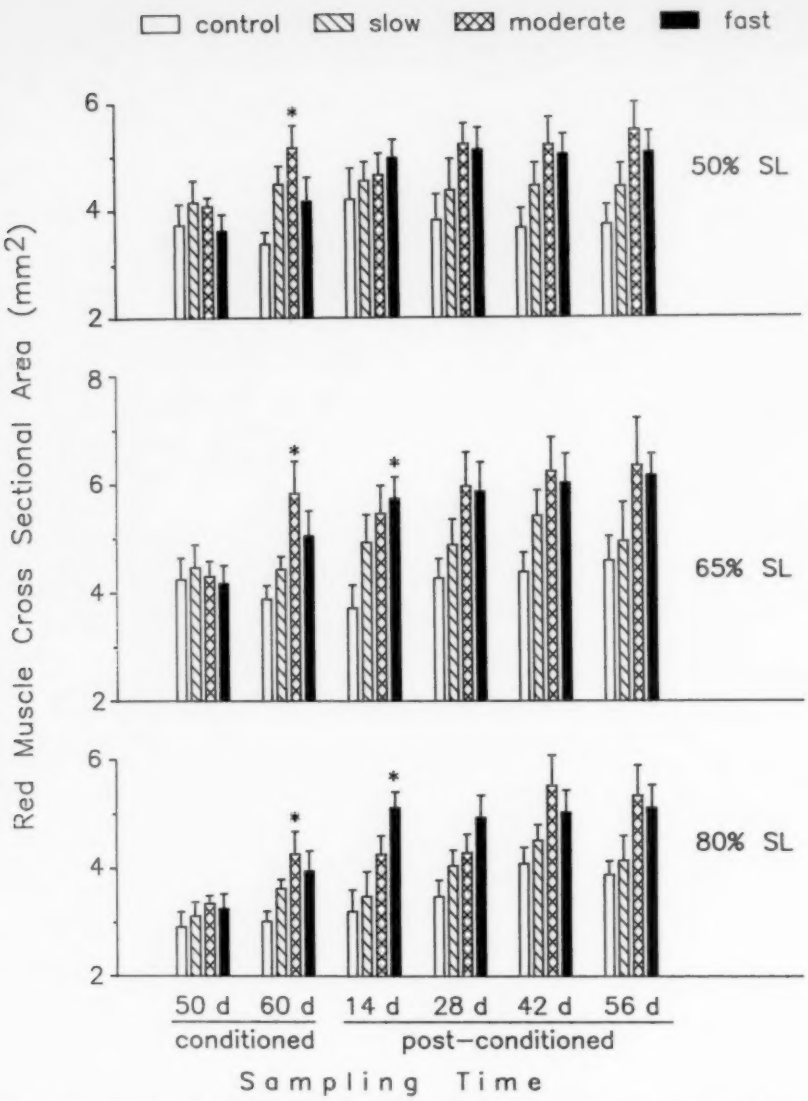

FIG. 2. Cross-sectional area of red muscle at 50, 65, and 80% SL of fish exercise conditioned at different flow velocities for 50 and 60 d and at 14, 28, 42, and 56 d post-conditioning (legends as in Fig. 1).

However, after a 60-d conditioning period, mean red and white muscle cross-sectional areas at all sections of the moderate velocity fish were significantly greater ($P < 0.05$) than those of the controls. After 14 d post-conditioning, mean red cross-sectional areas at 65 and 80% SL of the fast-velocity fish were significantly greater ($P < 0.03$) than those of the controls. After 14 and 28 d post-conditioning, mean white cross-sectional areas at all sections in the moderate- and the fast-velocity fish were significantly higher ($P < 0.05$) than in those of the controls.

After 60 d of conditioning, the red to white muscle ratio was uniformly higher ($P < 0.05$) in exercise-conditioned fish than in controls at all sections. However, after 14 d post-conditioning, only the fast-velocity fish had a significantly higher ($P < 0.05$) red to white muscle ratio than the controls, and only at 80% SL (Fig. 4).

Critical swimming velocities (U_{crit}) of fish showed a linear trend with increasing exercise-conditioning velocities (Fig. 5). Although the results in Fig. 5 are expressed in centimetres per second, conversion to body lengths per second does not change any of the significant relationships. After 50 d of conditioning, the fast-velocity fish had the highest mean U_{crit} which was significantly higher ($P < 0.01$) than that of the fish in other treatments. Moderate-velocity fish had significantly higher ($P < 0.03$) mean U_{crit} than the slow-velocity and control fish, while the slow-velocity fish had significantly higher ($P < 0.02$) mean U_{crit} than the controls. After 60 d of conditioning, the moderate- and fast-velocity

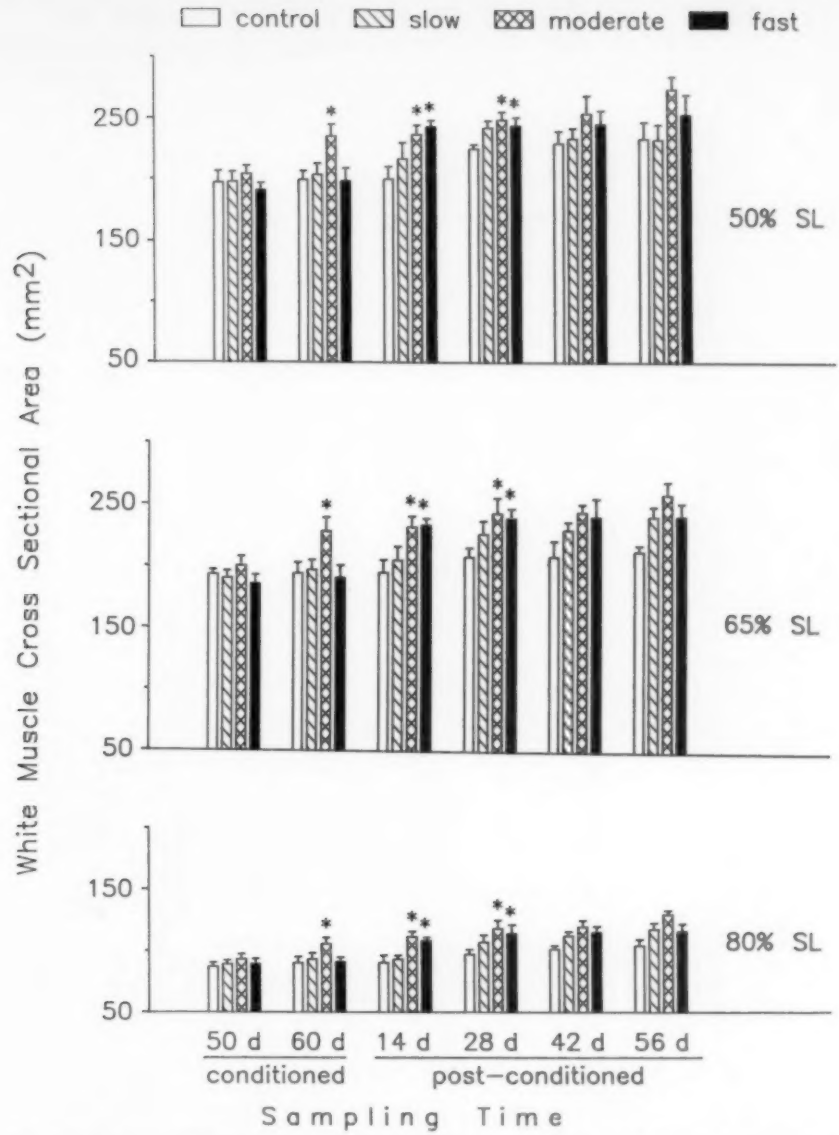

FIG. 3. Cross-sectional area of white muscle at 50, 65, and 80% SL of fish exercise conditioned at different flow velocities for 50 and 60 d and at 14, 28, 42, and 56 d post-conditioning (legends as in Fig. 1).

fish had significantly higher ($P < 0.01$) mean U_{crit} than the slow-velocity and control fish. After 14 and 28 d post-conditioning, the moderate- and fast-velocity fish had U_{crit} significantly higher ($P < 0.02$) than the controls. After 42 and 56 d post-conditioning, the fast-velocity fish had U_{crit} significantly higher ($P < 0.05$) than the slow-velocity and control fish.

Discussion

Growth and Muscular Development

Optimum exercise velocity for fish growth varies with species, e.g., 1.5 bl·s^{-1} for brown trout (*Salmo trutta*) (Davison and Goldspink 1977) and coho salmon (*Oncorhynchus kisutch*) (Besner 1980), 1–2.2 bl·s^{-1} for rainbow trout (Greer Walker and Emerson 1978; Nahhas et al. 1982a), 0.85 bl·s^{-1} for brook trout (*Salvelinus fontinalis*) (East and Magnan 1987), and 1.75 bl·s^{-1} for Arctic char (*Salvelinus alpinus*) (Christiansen and Jobling 1990). In our study, the optimum exercise velocity for growth in YOY striped bass was 1.5–2.4 bl·s^{-1} (Fig. 1). However, 60 d of exercise conditioning seemed to be required at this velocity to show any significant effects on body weights (Fig. 1). This difference in weight was maintained up to 56 d post-conditioning. Fish exercise conditioned at velocities lower (0.5–1.2 bl·s^{-1}) or higher (2.4–3.6 bl·s^{-1}) than the mod-

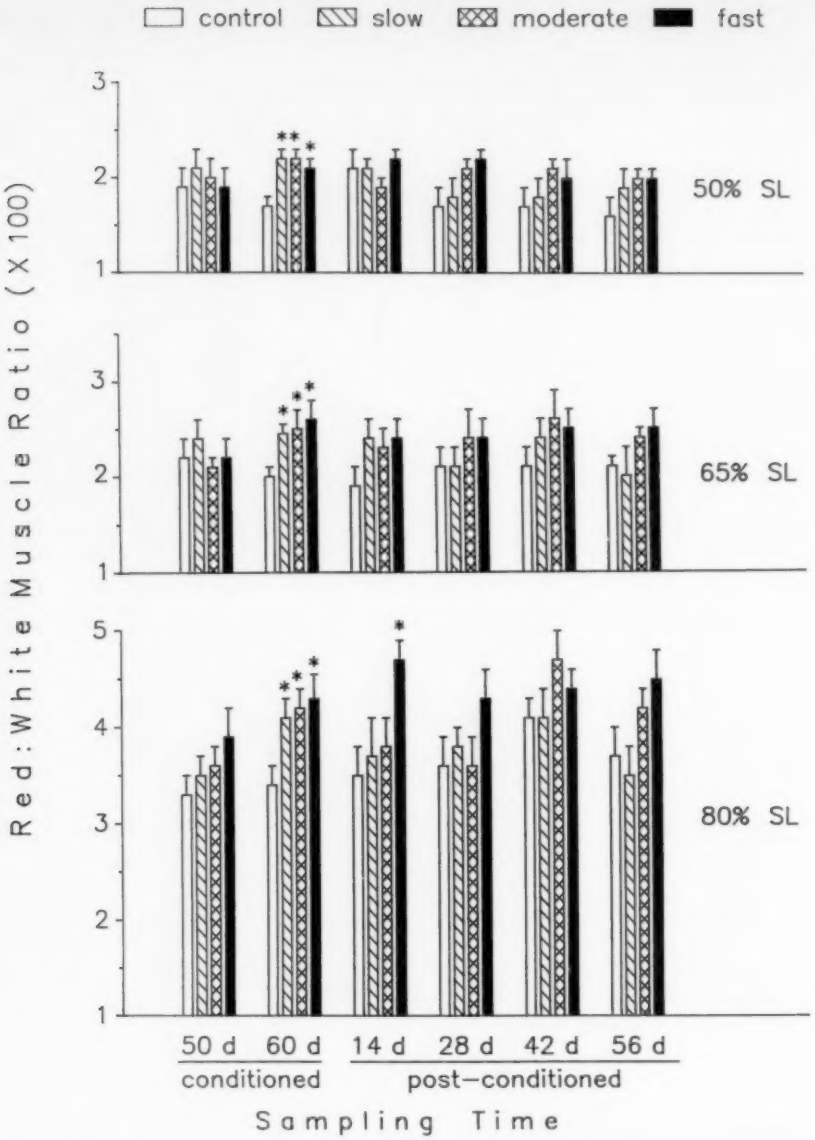

FIG. 4. Red to white muscle area ratio at 50, 65, and 80% SL of fish exercise conditioned at different flow velocities for 50 and 60 d and at 14, 28, 42, and 56 d post-conditioning (legends as in Fig. 1).

erate velocity grew more slowly than those exercise conditioned at the moderate velocity. This supports the concept that fish growth is generally maximized at intermediate swimming velocities (e.g., Davison and Goldspink 1977; Greer Walker and Emerson 1987; Nahhas et al. 1982a; Besner and Smith 1983; East and Magnan 1987; Christiansen and Jobling 1990). However, some species that are typically found only in still or slowly moving water (e.g., goldfish (*Carrasius auratus*)) often grow less at any swimming speed than the control (Davison and Goldspink 1978).

At slow velocity, the energy requirement for swimming is very low (Goldspink 1977). However, at higher velocities, energy turnover increases with swimming velocity (e.g., Beamish 1980). The higher energy cost of swimming contributes to the overall metabolic load and takes its toll on the growth of fish (e.g., Ware 1975; Davison and Goldspink 1978; White and Li 1985; Gamperl et al. 1988). In our study, exercise conditioning at the fast velocity (2.4–3.6 bl·s^{-1}) for 60 d did not affect body weight.

At 14 d post-conditioning, mean body weight of the fast-velocity fish was significantly higher than that of the control fish. Although we did not measure food consumption and food conversion rates, it is probable that these rates were proportionally higher in fish conditioned at faster swimming velocities (Davison and Goldspink 1977; Brett and Groves 1979; Christiansen and Jobling 1990; Boisclair 1992;

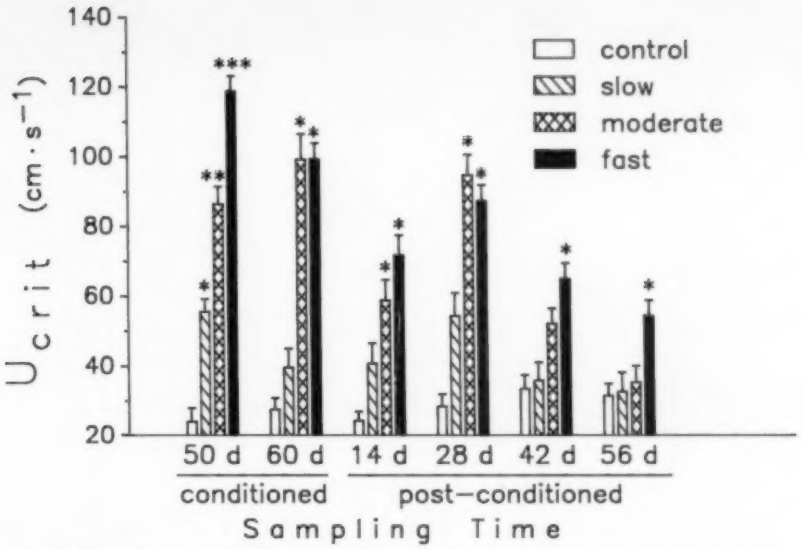

FIG. 5. Critical swimming velocity (U_{crit}) of fish exercise conditioned at different flow velocities for 50 and 60 d and at 14, 28, 42, and 56 d post-conditioning (error bar = SEM; n = 12; *significantly greater than the control; **significantly greater than * and control; ***significantly greater than **, *, and control; $P < 0.05$).

Christiansen et al. 1992). For the fast-velocity fish, increased food consumption may have continued after conditioning had stopped (overshoot). During this period, the "excess" ration may have been used for growth, similar to compensatory growth noted for other species when ad libitum rations were restored after food deprivation (Peter 1979; Blasco et al. 1992; Weiser et al. 1992; Mendez and Wieser 1993). We could not find other examples of "post-exercise compensatory growth" in the literature, and we believe it is an interesting area for future exercise-conditioning research.

Growth increases in fish exercise-conditioned at optimum velocity may be due to several interacting factors:(a) better food consumption and food conversion rate (Davison and Goldspink 1977; Christiansen and Jobling 1990; Boisclair 1992; Christiansen et al. 1992), (b) increased growth hormone levels (Barrett and McKeown 1988a, 1988b), (c) reduced antagonistic behavior (East and Magnan 1987; Christiansen et al. 1989; Christiansen and Jobling 1990), (d) reduced stress levels (Woodward and Smith 1985), and (e) increased protein synthesis rates (Houlihan and Laurent 1987). Young rainbow trout showed significant protein synthesis in the heart muscle and in the red and white muscles when exercised at 1 bl·s^{-1} for 6 wk. Although protein degradation was also faster in these tissues, the net protein gain was higher, especially in the red and white muscle tissues (Houlihan and Laurent 1987). Our previous study (Young and Cech 1993a) showed significant increases in red muscles at 80% SL and white muscles at all sections (50, 65, and 80% SL) in YOY striped bass exercise conditioned for 6 d at 1.2–2.4 bl·s^{-1}. Our present results (Fig. 2 and 3) also showed significant increases in the red and white muscles at all sections in YOY striped bass exercise conditioned for 60 d at 1.5–2.4 bl·s^{-1}. These increases in muscle tissues were isometric to body growth.

After 60 d of exercise conditioning, the red to white muscle ratios in all exercise-conditioned fish were significantly higher than in the controls (Fig. 4), indicating mostly aerobic muscular benefits of conditioning even at the fast velocity. Interestingly, the fast conditioning velocity range (2.4–3.6 bl·s^{-1}) encompasses the U_{crit} (2.90–3.28 bl·s^{-1}) measured on larger striped bass (24–29 cm SL) by Freadman (1979). However, the red to white ratio difference persisted up to 14 d post-conditioning only for the fast-velocity fish.

Swimming Performance

Fish make good research animals for exercise physiology (for reviews, see Hoar and Randall 1978; Blake 1983; Webb and Weihs 1983; Davison 1989) because most can be forced to swim at known speeds and many are responsive to exercise conditioning. The exercise-conditioning response is dramatic in terms of swimming performance (e.g., Farlinger and Beamish 1978; Broughton et al. 1980; Nahhas et al. 1982b; Besner and Smith 1983; Leon 1986; Houlihan and Laurent 1987; Pearson et al. 1990; Young and Cech 1993a). Coho salmon exercise conditioned for 40 d at 1.5 bl·s^{-1} showed increased swimming endurance that remained significantly higher than that of controls after 2 mo of rest (Besner and Smith 1983). In our study, improved swimming performance was shown after 50 d of exercise conditioning even at the slow velocity (0.5–1.2 bl·s^{-1}, Fig. 5). Those fish exercise conditioned at the moderate velocity (1.5–2.4 bl·s^{-1}) had even better swimming performance than those exercise conditioned at the slow velocity, and those exercise conditioned at the fast velocity (2.4–3.6 bl·s^{-1}) had the best swimming performance of all. This benefit persisted up to 28 and 56 d post-conditioning for the moderate- and fast-velocity fish, respectively.

It would be of interest in future studies to compare the swimming performance of consistently exercise-conditioned fish with that of intermittently trained fish. For example, brief exposure to periods of high velocity might improve swimming performance due to training, without concomi-

tant structural and physiological changes. The dramatic increase in swimming performance exhibited by 50-d conditioned fish at the fast velocity (Fig. 5) with no significant changes in size (Fig. 1), red muscle area (Fig. 2), or white muscle area (Fig. 3) may have resulted from swimming training ("learning") as well as from other structural or physiological changes which we did not measure.

Recommendations

Exercise conditioning has been recommended as an effective rearing strategy not only for commercial fish to increase economic gain (Totland et al. 1987) and to improve flesh texture (Tachibana et al. 1988), but also for fish used for mitigation purposes to increase survival and stamina (Horak 1972; Gatz 1973; Besner 1980; Besner and Smith 1983; Shchurov et al. 1986; Miklayhenko 1990), to increase the percentage return as spawners (Wendt and Saunders 1973), to increase recapture rates (Burrows 1969; Creswell and Williams 1983), and to increase growth, muscular development, and swimming performance (Young and Cech 1993a) and stress responses and recovery (Young and Cech 1993b) that could directly affect survival in natural environments.

For YOY striped bass used for mitigation purposes, we recommend exercise conditioning for at least 60 d at the intermediate velocity of 1.5–2.4 $bl \cdot s^{-1}$. This rearing regime increases red and white muscle tissues and increases swimming performance, with increases persisting up to 28 d post-conditioning. It also increases fish size persisting up to 56 d post-conditioning. We further recommend that YOY striped bass exercise conditioned at 1.5–2.4 $bl \cdot s^{-1}$ for 60 d be stocked 0–28 d post-conditioning to maximize conditioning benefits. If a fast velocity of 2.4–3.6 $bl \cdot s^{-1}$ is used for exercise conditioning of YOY striped bass for 60 d, we recommend that stocking be delayed until 14–28 d post-conditioning enhancing both size and swimming benefits.

The increased size and swimming performance of exercise-conditioned YOY striped bass should improve survival of fish stocked into natural environments. Increased size improves predation success and shortens vulnerability time as potential prey (Junge and Phinney 1963; Townsend and Calow 1981; Werner et al. 1983; Folkvord and Hunter 1986; Margulies 1990). Better swimming performance increases feeding efficiency, escape response, and maintenance of station against a current (Beamish 1978; Webb 1984, 1986). Stocking of exercise-conditioned YOY striped bass for mitigation purposes helps sustain a fishery by increasing the number of returning spawners to enhance natural reproduction.

Future research in developing large-scale systems for exercise conditioning YOY striped bass and in quantifying effects of returning spawner numbers would be valuable.

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Effects of Different Exercise Conditioning Velocities on the Energy Reserves and Swimming Stress Responses in Young-of-the-Year Striped Bass (*Morone saxatilis*)

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Young-of-the-year striped bass (*Morone saxatilis*) were exercise conditioned for 60 d at four different water velocities: <0.02 (control), 0.5–1.2 (slow), 1.5–2.4 (moderate), and 2.4–3.6 body lengths·s^{-1} (fast). Exercise conditioning at the moderate velocity increased body weight and standard length. Lipid contents and wet weight lipid percentages in the carcass were higher in the moderate- and fast-velocity fish than in the controls, and dry weight lipid percentages in the carcass and whole fish at the moderate velocity were higher than in those conditioned at the fast velocity. Moderate-velocity fish had resting (nonstressed) cortisol levels lower than those in the controls. After swimming stress, plasma cortisol, lactate, osmolality, and hematocrit levels in all fish were very high with no significant differences among treatments, while muscle pH values in all fish were lower than those in resting fish. Within the post-swimming stressed fish, muscle pH was lower in the controls than in those conditioned at the fast velocity. Overall, exercise conditioning in the moderate to fast velocity range increased body lipids and decreased post-swimming stress acidosis in young-of-the-year striped bass.

Des jeunes de l'année du bar d'Amérique (*Morone saxatilis*) ont été conditionnés à l'exercice pendant 60 jours à quatre vitesses de déplacement dans l'eau différentes : <0,02 (témoin), 0,5 à 1,2 (lente), 1,5 à 2,4 (modérée) et 2,4 à 3,6 longueur de corps·s^{-1} (rapide). Le conditionnement à l'exercice à la vitesse modérée a accru le poids corporel et la longueur normale. La teneur en lipides et le pourcentage en lipides du poids humide de la carcasse sont plus élevés chez les poissons conditionnés aux vitesses modérée et rapide que chez les poissons témoins, et le pourcentage de lipides en poids sec de la carcasse chez le poisson entier est plus élevé chez les poissons conditionnés à la vitesse modérée que chez les poissons conditionnés à la vitesse rapide. Chez les poissons conditionnés à la vitesse modérée, les concentrations de cortisol au repos (sans stress) sont inférieures à celles des poissons témoins. Après le stress induit par la nage, les teneurs en cortisol et en lactate du plasma, l'osmolalité et l'hématocrite chez tous les poissons sont très élevés, sans que l'on observe une différence significative entre les traitements; par contre, le pH musculaire chez tous les poissons est plus bas que celui des poissons au repos. Chez les poissons stressés après la nage, le pH musculaire est inférieur chez les poissons témoins à celui des poissons conditionnés à la vitesse rapide. Globalement, le conditionnement à l'exercice à une vitesse allant de modérée à rapide accroît la teneur en lipides de l'organisme et décroît l'acidose causée par le stress post-natatoire chez les jeunes de l'année du bar d'Amérique.

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California populations of striped bass (*Morone saxatilis*) have declined sharply since the mid-1970s (Stevens et al. 1985; Stevens 1992). From 1981 to 1991, about 11 million young-of-the-year (YOY) striped bass were stocked into the Sacramento – San Joaquin delta system for mitigation purposes by the California Department of Fish and Game (CDFG) (N. Villa, CDFG, personal communication). Theoretically, striped bass mitigation could be enhanced by stocking fish that are in better condition. Exercise conditioning may improve fish condition before their release in natural environments.

Exercise conditioning at water velocity of 1.2–2.4 body lengths (bl)·s^{-1} for 60 d has been shown to improve growth, muscular development, and swimming performance (Young and Cech 1993a) and handling stress responses and recovery (Young and Cech 1993b) in cultured and wild YOY striped bass. Their optimum exercise-conditioning velocity for growth and swimming performance was 1.5–2.4 and 2.4–3.6 bl·s^{-1}, respectively, with both effects persisting 56 d post-conditioning (Young and Cech 1994). However, the roles of exercise conditioning in the body condition of striped bass (energy reserves) as well as its ability to perform against a swimming challenge after stocking have been unclear.

In fish and other animals, excess calories are primarily stored in lipid (fat) depots when food intake is high. When food availability is low, energy metabolism switches from a balanced utilization of carbohydrate and fat to almost pure reliance upon lipid catabolism. The function of lipids as energy reserves of high energy content per unit weight is

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one of the most significant functions of these compounds in animals (Blaber 1975; Allen 1976). Thus, energy reserves in fish can best be calculated from measurements of neutral lipid content (Allen 1976). In striped bass stocked for mitigative purposes, energy reserves could be one of the major factors determining their survival, due to an expected time lag before they can start catching food efficiently. Ersbak and Haase (1983) reported that the eventual mortality of brook trout (*Salvelinus fontinalis*) was related to nutritional deprivation after stocking in streams. Energy reserves are necessary not only for standard and routine metabolism, but also for active metabolism used in maintaining position in or swimming against currents (Wakeman and Wohlschlag 1982; Brett 1983; Feldmeth 1983; Bernatchez and Dodson 1985; Calow 1985). It is important to know how exercise conditioning affects the energy reserves of fish used for mitigative stocking.

Appropriate stress responses regulate physiological systems and may improve survival after fish transport and stocking (Carmichael et al. 1984; Nikinmaa et al. 1984). For fish cultured in tanks or raceways with very slow or negligible water current, any subsequent current could represent another stress aside from transport and stocking stress. Zelnik and Goldspink (1981) showed that swimming, even at a slow speed of 1 $bl \cdot s^{-1}$ for 15 min, caused a significant increase in rainbow trout (*Oncorhynchus mykiss*) plasma cortisol. Ottaway and Clarke (1981) observed that some stocked young brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) were very vulnerable to downstream displacement by water flow. When released in natural environments, cultured YOY striped bass could find swimming against a tidal current quite stressful. Added to this stress of swimming against tidal currents is the need to escape predators or to catch food. It is therefore important to find out if swimming stress responses could be improved by exercise conditioning.

We wondered if exercise conditioning would increase energy reserves as well as improve physiological responses to swimming stress in YOY striped bass used for mitigative purposes. Specifically, we measured the effects of exercise conditioning at different velocities on their energy reserves and on their physiological responses when swimming over a long period of simulated tidal current followed by a short period of simulated burst swimming (as in predatory activity or escape response).

Methods

Methods and materials used in this study were similar to those used in the previous year's study (Young and Cech 1994). YOY striped bass were obtained from the CDFG Central Valley Hatchery in Elk Grove. Fish were held in the laboratory at the University of California, Davis, Institute of Ecology, for 2 wk in a 1 × 2.3 m fiberglass tank with a continuous flow of slightly chilled (21–24°C), air-equilibrated, nonchlorinated well water and continuous aeration. After a 1-d fast, fish were individually weighed in a pretared beaker of water and randomly distributed among 12 plastic raceways. Each raceway was a black ABS plastic tank measuring 150 cm long, 30 cm wide, and 25 cm deep. A screen was placed cross-sectionally 30 cm from each end, and a PVC sheet longitudinally divided the inner section into two compartments. Each compartment was stocked with 15 fish. A Teel submersible pump with an attached flow diffuser was placed beyond the screen at each end of the raceway to generate the desired water velocity. Each raceway had a water depth of 15 cm with a continuous flow of air-equilibrated well water at 2 $L \cdot min^{-1}$ creating a velocity of <1 $cm \cdot s^{-1}$.

The fish were subjected to four different water velocities: <1 (control), 2–6 (slow), 8–12 (moderate), and 12–18 $cm \cdot s^{-1}$ (fast). Teel submersible pumps of three different horsepowers (HP) were used to generate the different water velocities: 1/200 (slow), 1/35 (moderate and control), and 1/12 HP (fast). Each submersible pump had an attached flow diffuser (4 × 12 cm) to provide cross-sectionally uniform water velocity. Horizontally, however, water velocity was higher (e.g., 6 $cm \cdot s^{-1}$ for the slow velocity) near the pump and lower (e.g., 2 $cm \cdot s^{-1}$ for the slow velocity) farther from the pump, creating a velocity range within a compartment. Tanks for the controls had working pumps so that fish could be subjected to similar vibrations, noise, and/or heat generated by pumps in exercise-conditioned treatments. The water flow, however, was diffused and directed away from the fish compartments so that only a negligible flow was created. After 30 d, water velocity was increased to 8–12 (slow), 12–18 (moderate), and 18–34 $cm \cdot s^{-1}$ (fast) by either moving the pump nearer the fish compartment (moderate and fast velocities) or using a pump with more HP (slow velocity). This resulted in consistent water velocities of <0.02 (control), 0.5–1.2 (slow), 1.5–2.4 (moderate), and 2.4–3.6 $bl \cdot s^{-1}$ (fast).

Water velocity and water quality were monitored regularly. Water velocity was determined using a digital Marsh McBirney flowmeter, and no "dead" spaces were found within the rearing compartments of the exercise-conditioned treatments. Water temperature (YSI telethermometers, max:min thermometers), dissolved oxygen content (Nester DO meter), and ammonia content (Hach ammonia kit) were monitored in each tank three times a week to ensure uniformly high water quality in all tanks. Fish were kept in a 12 h light : 12 h dark photoperiod regime using an automatic timer.

Fish gradually habituated to conditioning over 3 d; thereafter, pumps were only stopped for feeding and tank maintenance. At the start of the conditioning, pumps were turned on only between 08:00 and 17:00 for 3 d, but were left on (except during feeding and cleaning) throughout the rest of the conditioning period (60 d total). Fish were fed with Silver Cup No. 2 trout pellets (Murray Elevators, Idaho) at about 7% body weight four times daily for the first 2 wk, three times daily for the next 2 wk, and twice daily for the rest of the rearing period. Pumps were turned off during feeding (allotted time = 20 min) and cleaning (20 min) for a total of 40 min per feeding and cleaning. Fecal matter and extra food were removed before the pumps were turned back on.

After a rearing period of 60 d, fish were tested for physiological responses to swimming stress. It took a period of 5 d to complete testing. Swimming stress was produced with a recirculating water flume incorporating a variable-speed motor. After 10 min, the fish was subjected to a water velocity of 10 $cm \cdot s^{-1}$ for 5 min, 40 $cm \cdot s^{-1}$ for 20 min (simulating tidal current), followed by 70 $cm \cdot s^{-1}$ for 5 min (simulating burst swimming for predatory activity or escape response). Immediately after swimming, the fish was removed quickly from the swimming chamber. Blood was immediately sampled by caudal transection (Steucke and Schoettger 1967; Houston 1990) and collected into heparinized capillary tubes. Immediately after blood collection, the head of the

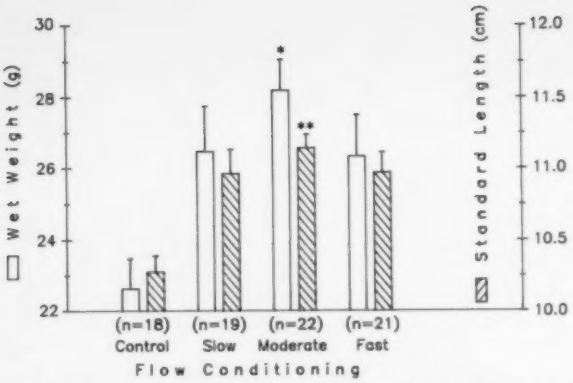

FIG. 1. Final wet weight and standard length of YOY striped bass exercise conditioned at different flow velocities for 60 d (mean ± SEM; *significantly higher than control, $P < 0.05$; **significantly higher than control, $P < 0.02$).

fish was cut off just behind the operculum, and the pH of white epaxial muscle on the right side was determined with an Orion (8163BN) combination pH electrode. Capillary tubes were centrifuged at 11 500 rpm for 5 min, hematocrit levels read, and plasma transferred to plastic freezer vials and frozen for later analyses. All parts of the fish were then placed in a labelled Whirl-pak container and frozen for lipid analysis.

Plasma was later thawed for osmolality, lactate, and cortisol measurements. Osmolality was measured using a calibrated Wescor 5100B (Logan, Utah) vapor pressure osmometer. Lactate levels were determined using a calibrated YSI (Yellow Springs, Ohio) model 27 lactate analyzer. Cortisol levels were measured using a modified enzyme immunoassay (ELISA) (Munro and Stabenfeldt 1985; Young and Cech 1993b).

Lipid contents were gravimetrically determined after moisture and subsequent ether extractions of thawed specimens to constant weights (Reznick and Braun 1987). Briefly, fish were thawed, dissected, and the body cavity contents (entrails) placed in separate 12-mL preweighed glass vials. Entrails included cavity fat deposits, liver, spleen, gallbladder, heart, kidney, swim bladder, and gastrointestinal tract (excluding contents). All head and body parts were cut into small sections (at most 1 cm^3), placed in preweighed 90-mL vials, and dried at 60°C to constant weight in a Precision vacuum drying oven. The samples in vials were then covered with anhydrous diethyl ether (Fisher Scientific, California) and the vials covered tightly and allowed to sit overnight in a fume hood. After several ether changes and drying at 60°C, the "lean" samples were reweighed. The lipid extraction, drying, and weighing processes were repeated with the ether replaced and poured off until a constant weight was obtained. Percentage lipid (% lipid) dry weight (d.w.) and % lipid wet weight (w.w.) were calculated as

$$\% \text{ lipid (d.w.)} = 100 \cdot (M1 - M2) \cdot M1^{-1}$$

$$\% \text{ lipid (w.w.)} = 100 \cdot (M1 - M2) \cdot M3^{-1}$$

where M1 = total dry weight (before ether treatment), M2 = lean dry weight (after ether treatment), and M3 = wet weight.

We used analyses of variance (ANOVA), Tukey's test, and regression analysis using SYSTAT software to test for significant differences among treatments and for correlation between flow and post-stress muscle pH. Cortisol values were transformed to $\log_{10}$ before conducting ANOVA and Tukey's test to increase homogeneity of variances. The value of 1 was added to each cortisol datum before log transformation to avoid negative values.

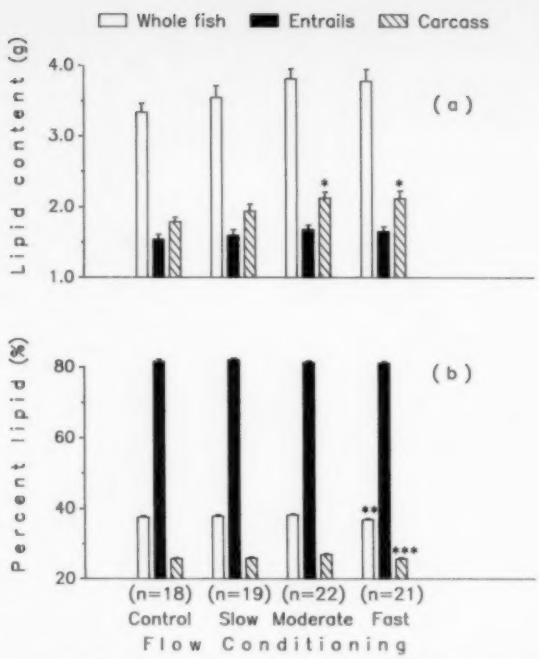

FIG. 2. Lipid weight and % lipid (d.w.) in whole fish, entrails, and carcass of YOY striped bass exercise conditioned at different flow velocities (mean ± SEM; *significantly higher than control, $P < 0.04$; **significantly lower than slow- and moderate-velocity fish, $P < 0.01$; ***significantly lower than moderate-velocity fish, $P < 0.02$).

Results

Temperature, dissolved oxygen, and ammonia contents of the water and fish mortality did not vary among different raceways. Daily mean temperature of the raceways fluctuated from a minimum of 21.0°C (±0.07 SE) to a maximum of 24.4°C (±0.07) with an overall mean temperature of 22.7°C (±0.09). Dissolved oxygen content remained high (7.8 ± 0.02 $mg \cdot L^{-1}$) and ammonia content remained low (0.69 ± 0.01 ppm) throughout the rearing period. No mortality occurred during the 60-d rearing period.

Control fish were observed to be randomly oriented within the compartment, but exercise-conditioned fish showed positive rheotaxis even at the slow-velocity treatment. Fish at fast velocities also exhibited a more synchronized locomotion.

Growth and Energy Reserves

YOY striped bass exercise conditioned for 60 d at the moderate velocity (1.5–2.4 $bl \cdot s^{-1}$) had significantly higher final wet weight ($P < 0.05$) and standard length ($P < 0.02$) than those of the controls (Fig. 1).

No significant differences were seen in the total lipid content of whole fish among different treatments, although moderate- and fast-velocity fish tended ($P = 0.05$) to have

TABLE 1. Mean (±SEM) plasma cortisol (ng·mL^{-1}), lactate (mmol·L^{-1}), osmolality (mosmol·kg^{-1}), hematocrit (%), and muscle pH of resting and stressed YOY striped bass reared at different flow velocities (*n* in parentheses).

	Control	Slow	Moderate	Fast
Cortisol				
Resting	26.9 ± 7.76 (20)	19.5 ± 7.29 (21)	3.2 ± 1.37[a] (18)	12.6 ± 4.63 (18)
Stressed	765.7 ± 47.83 (17)	760.9 ± 79.14 (15)	740.2 ± 36.59 (15)	782.5 ± 78.23 (13)
Lactate				
Resting	1.7 ± 0.06 (20)	1.9 ± 0.07 (22)	2.2 ± 0.15[b] (18)	1.9 ± 0.13 (18)
Stressed	26.2 ± 0.89 (18)	25.2 ± 1.24 (16)	25.1 ± 1.15 (16)	25.8 ± 1.43 (17)
Osmolality				
Resting	321.2 ± 1.99 (20)	323.8 ± 1.77 (21)	325.9 ± 2.01 (18)	323.7 ± 2.00 (18)
Stressed	363.2 ± 6.23 (17)	364.4 ± 5.42 (15)	369.4 ± 4.47 (15)	363.1 ± 5.22 (16)
Hematocrit				
Resting	35.8 ± 0.68 (20)	38.8 ± 0.88 (22)	36.9 ± 1.20 (18)	35.9 ± 0.86 (18)
Stressed	56.26 ± 2.25 (19)	54.8 ± 1.50 (17)	51.0 ± 1.17[c] (16)	54.6 ± 1.98 (17)

[a]Significantly lower than control ($P < 0.01$) and slow-velocity fish ($P < 0.05$).
[b]Significantly higher than control ($P < 0.01$).
[c]Slightly lower than control ($P = 0.07$).

higher total lipid contents. However, mean carcass lipid contents (Fig. 2a) were significantly higher ($P < 0.05$) in the moderate- and fast-velocity fish compared with controls. Entrails % lipid (d.w.) values were very high with no significant difference among treatments (Fig. 2b). Whole fish and carcass % lipid (d.w.) values at the fast velocity were significantly lower ($P < 0.04$) than those at the moderate and slow velocities. Carcass % lipid (w.w.) values were 6.4 ± 0.2, 6.8 ± 0.3, 7.9 ± 0.2, and 7.1 ± 0.2 for control, slow-, moderate-, and fast-velocity fish, respectively, with moderate- and fast-velocity fish having significantly higher ($P < 0.02$) % lipid (w.w.).

Stress Responses

YOY striped bass exercise conditioned at moderate velocity had a mean resting cortisol level significantly lower ($P < 0.01$) than that of the controls (Table 1). After swimming stress, all plasma cortisol levels were extremely high (740–782 ng·mL^{-1}), and no significant difference was seen among different treatments.

The mean [lactate] in resting moderate-velocity fish was slightly higher ($P < 0.01$) than that in the controls (Table 1). After swimming stress, plasma [lactate] in all fish was high (25–26 mmol·L^{-1}) with no significant differences among different treatments.

No significant differences in plasma osmolality and hematocrit were seen in resting YOY striped bass among treatments (Table 1). After swimming stress, plasma osmolality and hematocrit levels in all fish were high with no significant differences among treatments, although hematocrit in moderate-velocity fish tended ($P = 0.07$) to be lower than that in the controls.

Muscle pH of resting fish did not differ among treatments (Fig. 3), while after swimming stress, muscle pH decreased

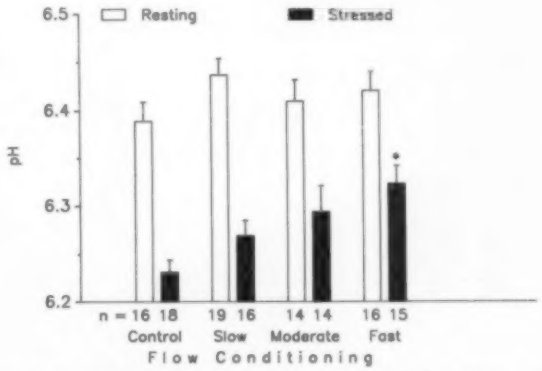

FIG. 3. Muscle pH in resting and stressed YOY striped bass exercise conditioned at different flow velocities (mean ± SEM; *significantly higher than control, $P < 0.01$).

($P < 0.01$) in all treatments. Post-swimming stress muscle pH was positively correlated with exercise-conditioning water velocities, and the mean muscle pH in the controls was significantly lower ($P < 0.04$) than that in the fast-velocity fish.

Discussion

Growth and Energy Reserves

Exercise conditioning for 60 d at moderate flow velocity (1.5–2.4 bl·s^{-1}) significantly improved growth in terms of body weight and standard length of YOY striped bass (Fig. 1). These observations confirmed our results from studies conducted in the previous year, i.e., optimum exercise-conditioning velocity for growth in YOY striped

bass was the moderate velocity (1.5–2.4 bl·s^{-1}; Young and Cech 1994). The effects of different flow velocities on growth of YOY striped bass have been discussed in detail in Young and Cech (1994).

Lipid storage in fish and in other animals is important because it represents a way of "buying a degree of independence from the environment" (Reznick and Braun 1987). In fish stocked for mitigative purposes, the amount of energy reserves could be a crucial factor for survival in the natural environment. Burrows (1969) observed that the survival of stocked chinook salmon (*Oncorhynchus tshawytscha*) fingerlings to adulthood was correlated with energy reserves at time of release. Peterson (1973) also correlated higher adult returns with high lipid content in Atlantic salmon smolts at time of release. The reduced overall condition in cultured salmonid fishes following their release (Reimer 1963; Carlander 1969; Hunt and Jones 1972) and the eventual mortality of fish were related to nutritional deprivation after stocking in streams (Ersbak and Haase 1983). During this period of starvation, stored lipid becomes the main energy source (Sherstneva and Shabalina 1973; Phleger 1988; Omino et al. 1991) not only for standard and routine metabolism, but also for active metabolism (Wakeman and Wohlschlag 1982; Brett 1983; Feldmeth 1983; Bernatchez and Dodson 1985; Calow 1985).

Our results showed that exercise conditioning for 60 d did not decrease the total or entrails lipid contents of fish (Fig. 2a). This indicates that with an ad libitum diet, exercise conditioning even at the fast velocity allows energy storage although energy cost increases with increased swimming speed (Freadman 1979). Moreover, whole-fish lipid contents in moderate- and fast-velocity fish tended ($P = 0.05$) to be higher than those in the controls, and the mean carcass lipid contents were significantly higher in the moderate- and fast-velocity fish than those in the controls. Although we did not measure lipid content of muscle tissues alone, carcass lipids were most probably mainly in muscle tissues. In Atlantic salmon raised in a swimming raceway for 8 mo, muscle tissues contained more glycogen and lipids than the fish reared in ordinary cages (Totland et al. 1987). Brown trout exercise conditioned at 1.5 bl·s^{-1} also showed increased muscle lipids (Love 1988). Davison and Goldspink (1977) reported the highest lipid build-up in brown trout exercise conditioned at 1.5 bl·s^{-1}, while those exercise conditioned at 3.0 bl·s^{-1} had intermediate lipid levels, and those exercise conditioned at 4.5 bl·s^{-1} had the lowest lipid levels. White and Li (1985) also observed that in juvenile chinook salmon, lipid storage was reduced with exercise at speeds greater than 1.9 bl·s^{-1}.

Brett (1965a) and Hanson et al. (1977) showed an energetic optimum at intermediate swimming speeds, while we showed an energetic optimum at intermediate exercise conditioning velocities. Brett (1965b) observed energetic optimality (i.e., gross cost of swimming decreased with increased swimming speed, reached a minimum at intermediate velocity, and then increased with further increases in swimming velocity) with respect to swimming costs for adult sockeye salmon (*Oncorhynchus nerka*) at approximately 50 cm·s^{-1} (1–1.5 bl·s^{-1}). The energy optimality apparently results in energy storage optimality. In our study, an exercise-conditioning velocity of 1.5–2.4 bl·s^{-1} was apparently energetically optimal for YOY striped bass. Whole-fish and carcass % lipid (d.w.) values in the moderate-velocity fish were significantly higher ($P < 0.04$; Fig. 2b) than those in the fast-velocity fish. Either less energy was stored as lipids or more lipids were directly used for exercise in YOY striped bass. Davison and Goldspink (1977) suggested that the reduced lipid in brown trout exercise conditioned at 3.0 bl·s^{-1} for 28 d could be due to the utilization of lipid and that lipid was the major fuel for swimming at this speed. Johnston and Moon (1980b) also observed that exercise conditioning enhanced the capacity for breaking down fatty acids in both white and dark muscles of pollock (*Pollachius virens*).

Carcass % lipid (w.w.) values in the moderate- and fast-velocity fish were significantly higher ($P < 0.02$) than those in the controls. Although the difference in % lipid (w.w.) seems small, it could be significant to the survival of fish after release into natural environments. Burrows (1969) observed over 100% greater adult returns in fall chinook salmon released at a carcass % lipid (w.w.) of 4.2% compared with fish released at 3.3%.

How the benefits of increased energy storage persist after termination of exercise conditioning constitutes an interesting question for future research on exercise conditioning. Our previous year's study (Young and Cech 1994) showed that increased growth in moderate-velocity fish persisted 56 d post-conditioning and that in fast-velocity fish, there was an increase in growth at 14 d post-conditioning.

Stress Responses

Exercise conditioning for 60 d at moderate velocity (1.5–2.4 bl·s^{-1}) resulted in significantly lower resting cortisol levels in YOY striped bass (Table 1). A similar observation was also reported in trained rainbow trout (Woodward and Smith 1985). Davison and Goldspink (1977) speculated that lower stress hormones (such as catecholamines and cortisol) were partly responsible for increased growth rates in exercise-conditioned brown trout. Interestingly, our previous study (Young and Cech 1993b) did not show a difference in resting cortisol levels between the controls and YOY striped bass exercise conditioned for 60 d at 1.2–2.4 bl·s^{-1}, a range that overlapped the slow and moderate ranges of the present study.

The plasma [lactate] in resting fish was consistently low. However, in moderate-velocity fish, the [lactate] was slightly, but significantly, higher than in the controls (Table 1). This result could be a sampling artifact because the moderate-velocity fish were feisty and struggled a lot when netted, likely increasing their plasma [lactate]. Broughton and Goldspink (1978) also reported slightly higher muscle [lactate] in resting roach (*Rutilus rutilus*) from running water compared with roach from still water, although Johnston and Moon (1980a) reported no significant difference in the resting plasma [lactate] between exercised (3 bl·s^{-1} for 21 d) and unexercised brook trout.

After swimming stress, plasma cortisol levels of all fish were extremely high and no significant differences were seen among treatments. Apparently, swimming at 40 cm·s^{-1} (3–4 bl·s^{-1}) for 20 min followed by swimming at 70 cm·s^{-1} (6–7 bl·s^{-1}) for 5 min proved to be very stressful for YOY striped bass. Zelnik and Goldspink (1981) reported lower cortisol elevations (~326 ng·mL^{-1}) in juvenile rainbow trout after 15 min of swimming at 5 bl·s^{-1}. Their values were similar to those in YOY striped bass 30 min after being subjected to handling stress (capture, net confining, and crowding; Young and Cech 1993b).

Plasma [lactate], osmolality, and hematocrit in all fish were high after swimming stress and there were no significant differences among treatments. The [lactate] values were higher than those in YOY striped bass subjected to handling stress (Young and Cech 1993b). The increased osmolality and hematocrit data indicated hemoconcentration from fluid shifts where water moves from plasma (concentrating plasma electrolytes, thereby increasing osmolality) into muscles and erythrocytes (swelling them slightly). The fluid shifts presumably resulted from transmembrane ions and subsequent water movements mediated by endogenous catecholamine secretions associated with the swimming stress (Eddy 1981; Nikinmaa et al. 1983; Wood and Perry 1985; Reid and Perry 1991). Nikinmaa et al. (1984) found adrenergic swelling in striped bass erythrocytes, increasing the hematocrit. Splenic contractions associated with exercise would also increase hematocrit (Yamamoto 1989; Wells and Weber 1990).

Muscle pH levels in all swimming-stressed fish were significantly lower than those in resting fish (Fig. 3). The La^- and H^+ may be of equal (Krebs et al. 1975; Jensen et al. 1983) or of different concentrations in the blood (Turner and Wood 1983). In fish, transfer of lactate from muscles to blood and subsequent removal from the blood is a fairly slow process (Jensen et al. 1983). In plaice (*Pleuronectes platessa*) recovering from strenuous exercise, the blood [lactate] values were lower in fish with prior exercise experience despite high muscle [lactate] (Wardle 1978). Our results showed that plasma [lactate] values were very high (Table 1) following strenuous exercise and that there were no significant differences in the [lactate] among treatments. However, mean muscle pH after swimming stress was lower in the controls than in the fast-velocity fish (Fig. 3). In addition, there was an increasing trend of post-swimming stress muscle pH with high exercise-conditioning water velocities ($r^2 = 0.43$; $P < 0.01$). This result suggests that exercise conditioning in YOY striped bass either produced less metabolic acid, increased H^+ washout, probably from enhanced perfusion associated with exercise conditioning, or increased muscle buffer concentrations. Hammond and Hickman (1966) showed that exercise conditioning in rainbow trout resulted in rapid removal of lactic acid from the muscle and blood when the fish became fatigued, resulting in less severe metabolic acidosis. Davie et al. (1986) showed increased numbers of capillaries allowing increased perfusion (thereby increasing H^+ washout) in both red and white muscle fibers in rainbow trout exercised for 200 d at 1 $bl \cdot s^{-1}$ compared with the controls. Love (1988) suggested that the higher pH in exercise-conditioned fish may be due to increased buffers, such as arsenine, in the fish muscle.

An interesting aspect for future research would be to track physiological/biochemical recovery from swimming stress among YOY striped bass exercise conditioned at different velocities. Exercise conditioning in YOY striped bass at 1.2–2.4 $bl \cdot s^{-1}$ for 60 d has been shown to improve stress responses and enhance recovery from handling stress (Young and Cech 1993b).

This study has shown that exercise conditioning at a moderate to fast velocity range causes small increases in body lipid and decreases a post-swimming stress acidosis in YOY striped bass. When released for mitigation purposes, exercise-conditioned YOY striped bass would have higher energy reserves and better swimming stress responses consistent with higher survival in natural environments.

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A Preseason Simulation Model for Fisheries on Fraser River Sockeye Salmon (*Oncorhynchus nerka*)

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Using a preseason fishery simulation model that refines previous reconstruction models, we explored the effects of various fishing regimes on annual catch and escapement of the major stocks of Fraser River sockeye salmon (*Oncorhynchus nerka*). To obtain harvest rate parameters for the model, we analyzed historical fishery catches to develop a theory of fish movement through time and space. Harvest parameters were then calculated using run reconstruction techniques. The model uses a daily time step to move fish sequentially through the major fishing areas. Fishing scenarios may be examined in the preseason to aid in developing annual management plans. Inputs of run size, timing, and diversion rate via Johnstone Strait are used to calculate run-timing curves which are normally distributed. The proportion of sockeye migrating via the northern approach or Johnstone Strait diversion, is shown to be a major factor in the sensitivity of fishing plans to altered input parameters. Clearly stated goals and objectives for spawning escapement and allocation of catch by stock groups are essential in the modelling process. The model permits managers to examine underlying assumptions and biological, economic, and social requirements centred on the harvest of these stocks.

À l'aide d'un modèle de simulation des pêches d'avant-saison amélioré par rapport au modèle de reconstitution précédent, nous avons étudié les effets de différents régimes de pêche sur les prises et les échappées annuelles des principaux stocks de saumon nerka (*Oncorhynchus nerka*) du fleuve Fraser. Afin d'obtenir les paramètres relatifs aux taux de récolte pour le modèle, nous avons analysé des prises historiques de pêche pour élaborer une théorie du mouvement des poissons dans le temps et dans l'espace. Les paramètres de récolte ont ensuite été calculés par des techniques de reconstitution de la montaison. Le modèle s'appuie sur un pas de temps journalier pour suivre le déplacement des poissons de manière séquentielle à travers les principales zones de pêche. Durant l'avant-saison, il peut être utile d'examiner les scénarios de pêche pour faciliter l'élaboration de plans de gestion annuels. On introduit la taille de la montaison, son moment et son taux de déviation à travers le détroit Johnstone pour produire des courbes d'analyse temporelle des montaisons normalement distribuées. Nous démontrons que la proportion des populations de saumon nerka migrant par la voie septentrionale ou le détroit Johnstone constitue un facteur crucial dans la sensibilité des plans de pêche aux paramètres d'entrée altérés. Dans le processus de modélisation, il est impératif de définir clairement les buts et les objectifs en ce qui concerne le nombre de saumons qui atteignent les frayères et l'allocation des prises par groupe de stocks. Le modèle permet aux gestionnaires d'étudier les hypothèses sous-jacentes ainsi que les exigences biologiques, économiques et sociales associées à la récolte de ces stocks.

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After spending 1–3 yr in the Gulf of Alaska, Fraser River sockeye salmon (*Oncorhynchus nerka*) start their return migration to the spawning grounds. From late June to early October, the sockeye migrate through the coastal waters of northern British Columbia, Vancouver Island, and the lower Fraser River (Fig. 1) where commercial gauntlet-type fisheries are directed on the returning salmon. These sockeye originate from as many as 40 stocks from tributaries of the Fraser River (Henry 1961). Fishery management of Fraser River sockeye has developed over the past 50 yr to achieve adequate numbers of spawners for each of the stocks and to distribute catch amongst the fishermen. Currently, management involves estimating the timing and abundance of the main sockeye populations using racial analysis and abundance estimation procedures (Woodey 1987). Ultimately, the manager must design fisheries to obtain the desired catch and spawning escapement for each stock. The model that we describe in this paper is used prior to the fishing season to examine how sequential fisheries exploit the stocks and to simulate fishery options that best meet the goals and objectives of management.

Three recent developments concerning the management of Fraser sockeye are notable. First, the capacity of the fishery has increased. This has been brought about by technological improvement of the fleet accessing the resource (Pearse 1982). Second, new sharing arrangements have been developed between Canada and the United States (PSC 1990a) and there have been changes in domestic allocation of catch within the two countries (PSC 1990b). Third, rebuilding of Fraser River sockeye stocks is planned to continue

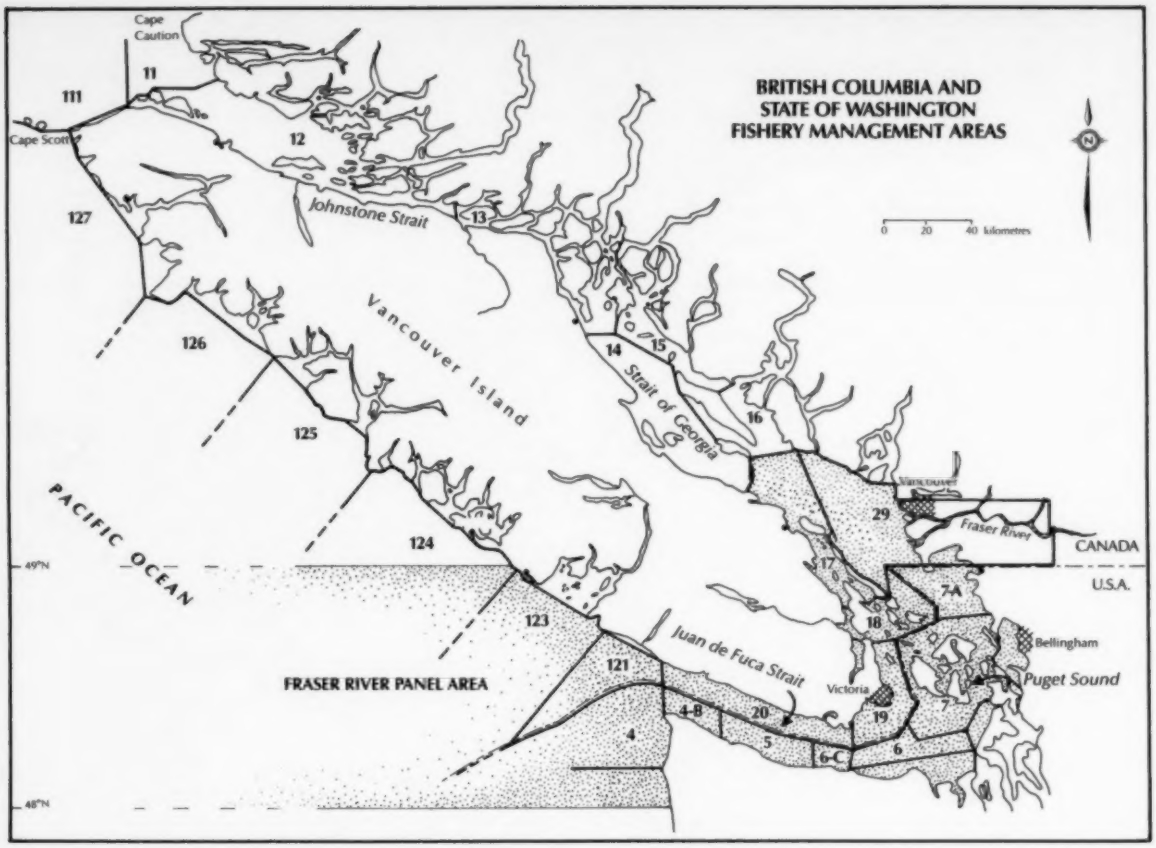

FIG. 1. Map of southern coastal British Columbia and northern Washington including fishing areas in which Fraser River sockeye are caught.

at an accelerated rate (DFO 1988). Initiated in 1937 by the Sockeye Salmon Fisheries Convention (IPSFC 1986), rebuilding of stocks progressed in the late 1970's when large returns permitted increased escapement.

These developments have resulted in a need to reduce harvest rate through an overall reduction in fishing time, which in turn has resulted in an increase in the complexity of management. Salmon management consists of two related activities: preseason planning and within-season adjustments to the preseason plan, based on revised information on the timing and abundance of the returning salmon stocks. While the in-season monitoring of stock sizes has developed over the years to its present level (Woodey 1987), the preseason planning of fisheries has not kept pace. Preseason plans were historically devised from a manager's experience, after a review of historical fisheries and predicted returning abundance. The development of new fishing objectives, catch allocations, and escapement regimes necessitates a more objective procedure for assessing fishing plans. The approach should logically involve simulation methods and an understanding of the theory of fish migration and exploitation.

The multi-time-period method of stock reconstruction described by Starr and Hilborn (1988) is applicable to salmon gauntlet fisheries that are typically scheduled over several weeks, in several fishing areas, and across several stocks. The data required by the method are catch by time and area, escapement by time and population, the residence time of each population in each harvest area, and the routing of the populations. The method may be used to estimate exploitation rate by stock by moving the fish backwards in time and space. However, Starr and Hilborn's notation does not provide for migration of fish into and out of fishing areas during the course of the fishery, nor does it provide for the distribution of the fleet in the fishing areas.

Run reconstruction methods can be used for forward simulations in time and space, under altered exploitation rates, for preseason planning. The application to preseason planning is particularly useful. Given preseason predictions of abundance, migration routing, timing, and fishery-specific harvest rates, the effects of various fishing regimes on catch and escapement can be investigated. The managers can then determine if and how stock-specific escapement goals and international and domestic allocations can be met.

In this paper, we describe a spreadsheet model that is designed to investigate the effects of various fishing regimes on catch and escapement. While the model has been designed for preseason planning of fisheries on Fraser River sockeye, the method has broader application for fisheries on other salmon stocks. We are unaware of the application of similar methods to other salmon management situations. We include the development of the theory used in the model and the application of that theory to the preseason planning

process. This theory includes a refinement to the Starr and Hilborn (1988) methodology to allow for migration of fish into and out of fishing areas during the course of the fishery and to take into account the distribution of the fleet in the fishing areas. We will justify the time step used in the model and describe the quantitative methods for removing catch from abundances entering a fishery area. Also, examples of fisheries examined by the model as well as results from a simulated management season are provided. Finally, we will discuss the validity of the assumptions in the model, the sources of input data, and the scope for use of the model.

Multi-Time-Period Model

Models of salmon movement dynamics for multiple time periods, stocks, and gauntlet fisheries have been described by several authors including Royce et al. (1963), Paulik and Greenough (1966), Palmer (1972), and Starr and Hilborn (1988). Starr and Hilborn presented their model as a backwards reconstruction model that can be run forward to assist in a planning process. However, the Starr and Hilborn model does not accommodate the movement of fish in an area during the fishery. The present model was initially designed as a forward planning model that we believe has application in backwards reconstruction. Our model represents a refinement to the Starr and Hilborn model to accommodate fish entering and exiting the area during the course of the fishery and the distribution of the fleet in the fishing areas.

We express space in units of time. The length of any fishing area is described by how much time is required for the fish to travel through the area. Similarly, we describe a group of fish comigrating during 1 d as a "daily migration block". Starr and Hilborn (1988) chose a half-weekly time step in their model. However, we chose a daily time step for two reasons. First, fish can travel through some fishery areas in a single day. These fishing areas require a daily time step for resolution. This is particularly important if we choose to model the effects of a fishery in one area on the fisheries in succeeding areas. Second, fisheries are managed by adding or removing days of fishing. The implications of such fishery adjustments can only be understood using a daily time step.

Not only do the fish move through the area, but the fleet moves as well. The distribution and movement of the fleet in an area affect the pattern of harvest of migration blocks. Movements of a fishing fleet are usually in response to depletion of available fish within an area during the fishery. At the opening of most fisheries, the fleet is spread throughout the fishing area. The fleet then moves to the entrance of the area to fish for incoming fish.

Our approach to the development of our model is to first place the traditional methods into a mathematically explicit format so that refinements can be built upon the basic model. For convenience, a list of the notation used is provided in the Appendix.

Temporal and Spacial Representation

A common assumption is that each fish in a stock migrates through a progression or gauntlet of fisheries and is resident in each fishery for a specified constant period. Also, all fish are equally vulnerable to fishing in any area, regardless of stock affiliation or if stock composition information is known. Let O_{sj} be the fishery identifier as stock s migrates through its jth area ($j = 1, 2, 3, \ldots, J_s$). Further, let r_{sf} be the residence (in days) of stock s in fishery f. The time taken by stock s to enter the first fishery (O_{s1}) and leave fishery f (w_{sf}) can then be expressed as

$$(1) \qquad w_{sf} = \sum_{j=1}^{f=O_{sj}} r_{s,O_{sj}}.$$

Thus, for example, if stock 1 migrated first through fishery 6, then through fishery 5, and finally through fishery 4 and the stock resided 1 d in fishery 6 and 2 d in each of fisheries 4 and 5, then the variables O, r, and w would have the following values:

$$O_{11} = 6 \quad r_{16} = 1 \quad w_{16} = 1$$
$$O_{12} = 5 \quad r_{15} = 2 \quad w_{15} = 3$$
$$O_{13} = 4 \quad r_{14} = 2 \quad w_{14} = 5$$

This specification of movement can be applied to either stock reconstruction, where harvest rates and initial run size are backcalculated from catch and escapement, or a preseason planning model in which catch and escapement are derived from assumed harvest rates and initial run size. The structure is said to be "rigid" because variable movement speeds are not accommodated (e.g., fish in the later part of a run may move at a faster speed). One alternative is to label the fish defined by a daily migration block (termed a "clump" of fish by Starr and Hilborn 1988) and specify the speed of each block with a calendar schedule. The bookkeeping task is greatly simplified with the assumption that each block can be treated independently in terms of both movement and harvest intensity. However, an important refinement that we wish to introduce is the alteration of harvest intensity based on the position of a block within a fishery and the possibility that a block could be vulnerable to more than one fishery during a day. Consequently, the blocks cannot be treated independently. For this reason, we preserve the traditional "rigid" structure depicted by equation (1).

Reconstruction Model

The reconstruction model estimates the harvest rate by fishery and day (h_{ft}) and the entering run size by stock and day (N_{st}) given the catch by day in each fishery (C_{ft}), the escapement of each stock by day (E_{st}), and the assumed movement of fish described by equation (1). The basic algorithm, which we will reference as model 1R, has the following six steps:

(A) Let

$$N_{st} = E_{s,t+\Delta t} \qquad \text{for all } s \text{ and } t \text{ where} \qquad \Delta t = w_{s,J_s}.$$

(B) Let $t = T$.

(C) Let

$$h_{ft} = \frac{C_{ft}}{C_{ft} + E_{ft}}$$

$$\text{for all } f \text{ where} \qquad E_{ft} = \sum_{s=1}^{n} \sum_{k=0}^{r_{sf}-1} N_{s,t-w_{sf}+k}.$$

(D) Let

$$N_{s,t-w_{sf}+k} = \frac{N_{s,t-w_{sf}+k}}{1-h_{ft}}$$

$$\text{for all } f, s, \text{ and } k = 0, 1, 2, \ldots, r_{sf} - 1.$$

(E) Let $t = t - 1$.

(F) If $t = 0$, then finished; otherwise, go to step (C).

Step (A) sets the run entering the first fishery to the escapement backdated by the time it takes for a stock to traverse all the fisheries. Step (B) sets the day subscripts (t) to the last day (T) in the season. The iterative part of the algorithm begins at step (C) where the daily harvest rate is calculated using the observed catch and the escapement from the fishery (E_{ft}, or the number of fish left in the fishery immediately after an opening). Note that the subscript k points to the migration blocks available to the fishery ($k = 0$ points to the block that is to leave the fishery during day t). Step (D) expands each of the migration blocks available to the fishery by the survival rate ($1 - h_{ft}$). Step (E) decreases the day subscript by 1 d in preparation for the next iteration and step (F) determines if all days have been processed.

Preseason Planning Model

The preseason planning model is the inverse of the reconstruction model. Catch and escapement are derived from assumed harvest rates, entering run pattern and fish movement described by equation (1). The basic algorithm, which we will reference as model 1P, has the following six steps:

(A) Let

$$N_{st} = \frac{N_s}{\sigma_s \sqrt{2\pi}} \exp\left\{ -\frac{1}{2}\left(\frac{t - t_{\max_s}}{\sigma_s} \right)^2 \right\}$$

for all s and t.

(B) Let $t = 1$.

(C) Let

$$C_{ft} = \sum_{s=1}^{n} \sum_{k=0}^{r_{sf}-1} h_{ft} N_{s,t-w_{sf}+k} \qquad \text{for all } f.$$

(D) Let $N_{s,t-w_{sf}+k} = (1 - h_{ft}) N_{s,t-w_{sf}+k}$

for all f, s, and $k = 0, 1, 2, \ldots, r_{fs} - 1$.

(E) Let $t = t + 1$.

(F) If $t = T$, then finished; otherwise, go to step (C).

Step (A) sets the entering run pattern of a stock, where N_s is the total run entering (return) of stock s, to a normal distribution over time with mean at the peak time ($t_{\max_s}$) and a stock-specific deviation (σ_s). Since a typical run is broken into about 40–70 daily blocks, a rectangular approximation for the area under the normal distribution has accuracy sufficient for modelling purposes. The mean (peak) and the deviation can be estimated from the entry timing curves generated from stock reconstruction. It is sometimes convenient to express (estimate) the deviation in terms of the duration or spread of the run and how much of the run the spread comprises, i.e.,

$$(2) \qquad \sigma_s = \frac{D_s}{2Z_\alpha}$$

where D_s is the duration or spread of the run in days and Z_α is the standard normal value at a confidence level of $1 - \alpha$. An alternative for the entry timing curve for the planning model is simply to use the timing curve obtained from stock reconstruction. However, these curves typically contain spikes and gaps where fish movement and fishery dynamics are not accurately captured by the reconstruction model. For planning purposes, we believe it is better to use a smoothed curve. Step (B) sets the day subscript (t) to the first day. The iterative part of the algorithm begins at step (C) where the catch is calculated from each block in the fishery using the assumed harvest rate. Step (D) reduces each of the migration blocks available to the fishery by the survival rate ($1 - h_{ft}$). Step (E) increases the day subscript by 1 d in preparation for the next iteration and step (F) determines if all days have been processed.

Refinements to the Multi-Time-Period Model

The multi-time-period model presented above is overly simplistic, as it does not adequately represent either the movement of fish in the area during a fishery or the fleet dynamics within the area. The importance of modelling the movement of fish into, and out of, an area during the fishery cannot be overemphasized. This is critical for several fisheries on Fraser sockeye that are small in area (containing 1 d of fish migration) but high in harvest rate (e.g., Canadian Area 20). Fish migrate through these areas in 1 d; however, over a 24-h period, fish that were in the area at the commencement of fishing are replaced by a new group of fish. Therefore, not one, but two daily migration blocks of fish are affected during the 24-h opening. In these situations, the second and third days of the fishery usually result in substantially less catch than the first day of fishing.

The number of refinements or expansions to the basic model is not limited by any specific fishery. Here, we present three refinements that model the observed behaviour of catch and are practical to implement. Since, for this paper, we are primarily interested in the preseason model, we restrict the mathematical development to model 1P and consider the reconstruction problem less formally. In general, our approach is to adjust the daily harvest rate within a fishery (h_{ft}) for fish that are only partially exposed to the fishing fleet, changes in fleet size, and spatial allocation of the fleet over time within the fishing area.

Refinement 1: Partial Exposure to Fishing

The basic model now takes a constant fraction from each block in the fishery, i.e.,

$$C_{ftsk} = h_{ft} N_{s,t-w_{ft}+k}$$

where k points to the daily migration block in the fishery. To simplify discussion in this section, we drop all subscripts and refer to the above relationship simply as

$$(3) \qquad C = hN.$$

In reality, fish that enter or leave a fishery during a day are only partially exposed to fishing. In other words, the block ($k = 0$) and the block entering ($k = r_{sf}$) the fishery should have a reduced harvest rate in comparison with the other blocks that remain in the fishery.

Conceptually, the fish partially exposed can be partitioned into fish that are at risk (N_C) and those that are not (N_E), i.e.,

$$(4) \qquad N = N_C + N_E.$$

Therefore, the catch must be

$$(5) \qquad C = hN_C.$$

We need to equate the fish that are not at risk with some function of harvest rate and fish that are at risk. The function should have the following intuitive characteristics: (1) $N_E = N_C$ as h approaches 0, (2) $N_E < N_C$ for $0 < h < 1$, and (3) $N_E = 0$ as h approaches 1. We first assume a general function that satisfies these characteristics:

$$(6) \quad N_E = (1 - h)^x N_c$$

for some $x > 0$. Using equations (3)–(6), N_E and N_C can be eliminated to obtain the result

$$(7) \quad C = \frac{hN}{1+(1+h)^x}$$

for the catch of fish entering or exiting the fishery. The value of x controls the contrast in harvest rate upon fish resident in the fishery for the entire day and those that are partially exposed to fishing. For small x the harvest rate on the partially exposed fish will be near half that of the resident fish. On the other hand, for large x, there will be little difference, so large values for x should not be considered ($x \leq 1$, say). We assume $x = 0.5$ to define the partially exposed harvest rate (u) as

$$u = 1 - \sqrt{1-h}$$

or the inverse

$$(8) \quad h = 2u - u^2 = u + u(1 - u).$$

Equation (8) suggests the useful concept of u as the elementary harvest rate of the partially exposed block with the block resident in the fishery fished twice at rate u. In other words, the partially exposed block is only available to the fishery for 50% of the day; hence, $x = 0.5$.

We can now apply equation (8) to the basic preseason planning model (1P) to obtain a new model (2P) for any stock s:

(A) Let

$$N_{st} = \frac{N_s}{\sigma_s \sqrt{2\pi}} \exp\left\{-\frac{1}{2}\left(\frac{t - t_{\max_s}}{\sigma_s}\right)^2\right\} \qquad \text{for all } t.$$

(B) Let $t = 1$.

(C) Let $j = 1$.

(D) Let $f = O_{sj}$.

(E) Let

$$C_{fts} = \sum_{k=0}^{r_{sf}} h_{ftk} N_{s,t-w_{sf}+k}.$$

(F) Let

$$N_{s,t-w_{sf}+k} = (1 + h_{ftk}) N_{s,t-w_{sf}+k} \qquad \text{for } k = 0, 1, 2, \ldots, r_{fs}.$$

(G) Let $j = j + 1$.

(H) If $j \leq J_s$, then go to step (D).

(I) Let $t = t + 1$.

(J) If $t = T$, then finished; otherwise, go to step (C).

Here,

$$(9) \quad h_{ftk} = 2u_{ft} - u_{ft}^2 \qquad \text{if } 0 < k < r_{fs}$$
$$= u_{ft} \qquad \text{otherwise.}$$

Note that in the basic model described by 1P, all stocks are processed each day and fisheries are processed in any order because one day's migration block can only exist in one fishery. The new model (2P), however, must process each stock through the sequence of fisheries on the migration route because a block entering a fishery ($k = r_{sf}$) could have been exposed to the previous fishery on the same day. The added steps (C), (D), (G), and (H) in the algorithm are simply to specify the required sequence of fisheries. After all calculations have been completed, the catch by fishery and day can be obtained by summing over all stocks:

$$C_{ft} = \sum_{s=1}^{n} C_{fts}.$$

Refinement 2: Fleet Distribution within an Area

The fleet is usually not distributed uniformly within a fishing area. Indeed, it is quite common for the fleet to redistribute effort in response to changes in fish abundance from one period to the next. We have divided a fishery into blocks to correspond to the time taken by a fish to traverse the area (r_{fs}) plus one more block to represent the fish migrating into the area on a given day. Differences in fishing intensity between the blocks can be depicted simply as a set of weights p_{ftk} ($k = 0, 1, 2, \ldots, r_{fs}$) specific to fishery f on day t scaled to 1. Estimates for these weights were simply derived from boat counts in each migration block obtained from aerial overflights during an opening. The weights notation for fleet allocation can now be incorporated into the harvest rate on each migration block (h_{ftk}) in equation (9) to obtain

$$(10) \quad h_{ftk} = p_{ftk}u_{ft} + p_{ft,k-1}u_{ft}(1 - p_{ftk}u_{ft}) \qquad \text{if } 0 < k < r_{fs}$$
$$= p_{ftk}u_{ft} \qquad \text{otherwise.}$$

Note that for the migration blocks resident in the fishery for the entire day ($0 < k < r_{fs}$) the fleet weight for both the current block (k) and the adjoining block ($k - 1$) into which the fish travel on day t is included.

Refinement 3: Fleet Size

The elementary fishing rate (u_{ft}) remains constant over all migration blocks within a fishery for a particular day. This rate can be directly related to the size of the fishing fleet (F_{ft}) with the catchability coefficient (q_f) as the constant of proportionality, i.e.,

$$(11) \quad u_{ft} = q_f F_{ft}.$$

For situations in which u saturates with increasing fleet size the following relationship can be used:

$$(12) \quad u_{ft} = a_f + b_f \ln(F_{ft})$$

where a_f and b_f are constants specific to each fishery. Some fisheries (e.g., Johnstone Strait) are fully saturated with fishing effort and a constant u can be used over all time.

Refinement 4: Reconstruction

While the focus of this paper is on the preseason planning model, analysis of historical data to estimate harvest rates and to examine the suitability of migration routing and timing influences the choice of input values used in the planning model. The refinements to the basic model introduced above add considerable complexity to the stock reconstruction algorithm. The preseason model described by P2 cannot be made to run in reverse because each stock is

processed separately (C_{ft} is the observed catch and not C_{fts}). However, under the condition that a migration sequence of fisheries could be specified common to all stocks, then a reconstruction model can be formulated with some additional notation. Let O_j be the fishery identifier as any stock moves through the jth area ($j = 1, 2, 3, \ldots, J$). The model can now be written as

(A) Let

$$N_{st} = E_{s,t+\Delta t} \qquad \text{for all } s \text{ and } t \text{ where} \qquad \Delta t = w_{s,J_s}.$$

(B) Let $t = T$.

(C) Let $j = J$.

(D) Let $f = O_j$.

(E) Solve

$$C_{ft} = \sum_{s=1}^{n} \sum_{k=0}^{r_{sf}} \frac{h_{ftk}}{1-h_{ftk}} N_{s,t-w_{sf}+k} \qquad \text{for } u_{ft}.$$

(F) Let

$$N_{s,t-w_{sf}+k} = \frac{N_{s,t-w_{sf}+k}}{1-h_{ft}}$$

for all s and $k = 0, 1, 2, \ldots, r_{sf} - 1$.

(G) Let $j = j - 1$.

(H) If $j \geq 1$, then go to step (D).

(I) Let $t = t - 1$.

(J) If $t = 0$, then finished; otherwise, go to step (C).

Here, the harvest rate on each block (h_{ftk}) is a function of u_{ft} defined by equation (10). In step (E), the escapement for each migration block is divided by the survival rate and multiplied by the harvest rate to obtain catch. The only unknown in step (E) is u_{ft} and a numerical solution can be found easily with the bisection method for each fishery and day (the desired solution for u_{ft} is real and bounded by 0 and 1).

Starr and Hilborn (1988) recommend pooling time periods to overcome problems in misallocation of catch by time period and further stated that a daily time step should be avoided because of this type of error. However, instead of slicing both catch and escapement into uniform daily intervals, reconstruction can proceed with catch accumulated over any time period, m (e.g., week, month), while escapement is maintained at a daily resolution under the assumption that u is constant over the time interval m. Thus, we can substitute step (E) with the following:

Solve

$$C_{fm} = \sum_{t \subset m} C_{ft} = \sum_{t \subset m} \sum_{s=1}^{n} \sum_{k=0}^{r_{sf}} \frac{h_{ftk}}{\prod_{t \subset m}(1-h_{ftk})} N_{s,t-w_{sf}+k}$$

for u_{fm}

where all variables are defined as above. Again, the only unknown is u_{fm} and a numerical solution can be found easily with the bisection method.

The reconstruction requires a migration sequence of fisheries common to all stocks (a stock need not be present in all fisheries). Violation of this condition requires first that stocks travel in opposite directions between fisheries and second that the catch taken in the interval t or m be from fish susceptible to capture in at least two of the fisheries in conflict. For Fraser River sockeye, the assumption is not violated. However, in theory, a set of simultaneous equations could be developed and solved for this situation. In practice, violations are rare and a good approximation can be obtained by ignoring any conflicts or through the specification of travel time between the fisheries in conflict such that the fish susceptible to capture do not overlap in the fisheries.

Examples of Fishery Simulations

The major fisheries on Fraser sockeye are located in southern British Columbia and Puget Sound south of Cape Caution (latitude 51°10′, Fig. 1). On the west coast of Vancouver Island, in Canadian (Pacific Fishery Management) Areas 121–127 and in Area 111 and 12, a troll fishery is operated, referred to as the outside troll fishery. In Areas 12–13 (Johnstone Strait), 16 (Sabine Channel), and 20 (Juan de Fuca Strait), net fisheries are operated consisting of purse-seine and gillnet vessels. A small gillnet fishery is operated in Area 11 following the same gillnet fishing schedule as in Area 12. Gillnet fisheries are operated in the Fraser River area proper and Gulf of Georgia, designated as Area 29. A small-scale troll fishery is operated in Canadian Areas 12–18 and in the marine portion of Area 29 and is referred to as the inside troll fishery. In Washington State, Fraser sockeye are caught in a limited gillnet fishery in United States (Puget Sound Salmon Management and Catch Reporting) Areas 4B, 5, and 6C (U.S. Juan de Fuca Strait) and a combined gillnet, purse-seine, and reef-net fishery operated in Areas 6 and 7 (Salmon Banks) and in Area 7A (Point Roberts). Separate fisheries are scheduled for United States Treaty Indian and Non-Indian fishermen. For a more detailed review of the features of these fisheries, the reader is referred to PSC (1991).

These fisheries can be modelled according to the various refinements of the multi-time-period model. Movement of the fleet typically occurs in fishing areas where the harvest rates are high and the boats move to take advantage of fish entering the area. Below, we apply equation (10) to each of the net fisheries (Table 1).

United States Area 7 and Canadian Area 13

These areas hold at any given time 2 d of migration or two migration blocks. As the fishery opens, two blocks are exploited. At the end of a single day of fishing, one of these blocks has left the area, one block continues to remain, and a third and new block of fish has entered the area. Therefore, three blocks of fish are affected by a 1-d fishery. Aircraft surveillance indicates that the fleet is spread evenly throughout the area, so each migration block is assumed to have equal allocation of fishing effort (Table 1a).

Canadian Area 12

Area 12 fisheries are more complex for two reasons. First, early in the season, the upper portion of the area is often closed, affecting the amount of fish exposed to fishing effort. Second, when the whole area is available to the fleet, the fleet moves to fish on unexploited fish migrating into the area as the fishery progresses.

When only the lower portion of Area 12 is open (when the so-called Lewis Point boundary is in force), only a single day

TABLE 1. Examples of exploitation patterns applied to each of the net fisheries. The harvest pattern (as a function of the elementary harvest rate (u) and harvest rate (h)) is obtained from the application of equation (10) for a specific migration block (k) with the associated effort allocation (the p's within the fishery). The subsequent catch taken from the migration block is also calculated.

Entering date	Sockeye abundance	Comment	Migration Pointer (k)	Effort allocation (p)	Harvest pattern	Harvest rate (%)	Catch
		(a) United States Area 7, 1-d fishery on Aug. 3					
Aug. 1	10 000	Leaving the area	0	1	u	50	5 000
Aug. 2	15 000	Remaining in the area	1	1	$2u - u^2$	75	11 250
Aug. 3	20 000	Entering the area	2	1	u	50	10 000
		(b) Canadian Area 12, 1-d fishery on Aug. 2; Lewis Point boundary in effect					
Aug. 1	10 000	Leaving the area	0	1	u	40	4 000
Aug. 2	15 000	Entering the area	1	1	u	40	6 000
		(c) Canadian Area 12, 1-d fishery on Aug. 4					
Aug. 1	10 000	Leaving the area	0	1	u	50	5 000
Aug. 2	15 000	Remaining in the area	1	1	$u + (u - u^2)$	75	11 250
Aug. 3	20 000	Remaining in the area	2	0.5	$1.5u - 0.5u^2$	62.5	12 500
Aug. 4	25 000	Entering the area	3	0.5	$0.5u$	25	6 250
		(d) United States Area 7A, 1-d fishery on Aug. 2					
Aug. 1	10 000	Leaving the area	0	2	$2u$	80	8 000
Aug. 2	15 000	Entering the area	1	1	u	40	6 000
		(e) United States Area 7A, 2-d fishery on Aug. 2					
Aug. 1	10 000	Leaving the area	0	2	$2u$	80	8 000
Aug. 2	15 000	Entering the area	1	2	$2u$	80	12 000
Aug. 3	20 000	Entering the area	2	1	u	40	8 000
		(f) Canadian Area 20, 1-d fishery on Aug. 2					
Aug. 1	10 000	Leaving the area	0	1	u	40	4 000
Aug. 2	15 000	Enteringthe area	1	2	$2u$	80	12 000
		(g) Canadian Area 20, 1-d fishery on Aug. 2					
Aug. 1	10 000	Leaving the area	0	1	u	40	4 000
Aug. 2	15 000	Entering the area	1	2	$2u$	80	12 000
Aug. 3	20 000	Entering the area	3	2	$2u$	80	16 000
		(h) Canadian Area 29, 1-d fishery on Aug. 2					
Aug. 1	10 000	Leaving the area	0	1	u	92.5	9 250
Aug. 2	15 000	Remaining in the area	1	1	u	92.5	13 875
Aug. 3	20 000	Entering the area	2	0	$0u$	0	0

of fish migration is available to the fishery. A 1-d fishery operates on two migration blocks with the fleet distributed evenly throughout the area open to fishing (Table 1b). When all of Area 12 is open, 3 d of migration are available for harvest at any time. The fleet is distributed such that 20% operates in the top third of the area, 40% in the middle third, and 40% in the bottom third. This represents a compromise between the actual fleet pattern early in the fishery (20–20–60%) and a redistribution of fleet effort during the second day (20–50–30%). The compromise situation is illustrated in Table 1c.

United States Area 7A

The gear distribution information collected during aerial surveys of this fishing area indicates that the fishing fleet is principally concentrated in the upper half of the area, presumably due to the presence of better fishing locations in that portion. Fishing scenarios are modelled to take these observations into account and follow the general rule that blocks of fish leaving the area are fully harvested at twice the rate as those fish entering the area (Table 1d).

Canadian Area 20

The Area 20 fishery is the reverse situation to that of United States Area 7A, since a line of boats rapidly forms at the entrance to the fishing area. As the fishery opens, a number of small "lineups" of boats are already in place to "clean up" the fish already in the area (the previous day's migration) in addition to the main line of boats at the entrance to the area. As the fishery continues, the boats move to the

TABLE 2. Summary of predictions by stock for abundance of adult returns, Johnstone Strait diversion rate, timing, and spread.

	Early Stuart	Early miscellaneous	Horsefly/ late Stuart	Chilko Stellako	Birkenhead	Adams/ late runs	Total run
Total run	125 000	931 000	2 000 000	1 450 000	1 125 000	10 769 000	16 400 000
Diversion (%)[a]	15	15	20	25	25	25	24
Diversion (%)[b]	15	35	52	52	58	58	55
Peak timing of run entering area							
Area 127	June 27	July 22	July 25	July 29	Aug. 7	Aug. 12	
Area 20[c]	July 2	July 27	July 30	Aug. 3	Aug. 15	Aug. 20	
Area 11	June 26	July 21	July 24	July 28	Aug. 6	Aug. 11	
Area 12[d]	June 29	July 24	July 27	Aug. 2	Aug. 11	Aug. 16	
Spread	25	35	30	30	25	25	

[a]Simulation 1.
[b]Simulation 2.
[c]First southern approach net area.
[d]First northern approach net area.

TABLE 3. Summary of routes, and days of travel from Area 127 to enter each area by stock group. Summer-run stocks include early Stuart, the early miscellaneous stock grouping, the Horsefly/late Stuart stock group, and the Chilko/Stellako stock group. Birkenhead is a stock with a migration pattern intermediate between summer-run stocks and the late-run stocks. Late-run stocks include the Adams/Lower Shuswap and the late miscellaneous group of stocks.

	Summer-run Stocks	Birkenhead Stock	Late-run Stock
Southern approach			
Area 127	0	0	0
Area 124	3	4	4
Area 20	5	7	7
Area 4B	6	8	8
Area 7	7	10	10
Area 7A	9	12	12
Area 29B	10	13	13
Area 29D	11	20	—[a]
Northern approach			
Area 11	−1	−1	−1
Area 12	2	4	4
Area 13	5	7	7
Area 16	8	10	10
Area 29B	10	12	12
Area 29D	11	19	—[a]

[a]Late-run stocks delay in the Gulf of Georgia for 4 wk before migrating upstream. This upstream migration is not modelled.

main line and work exclusively on the in-migrating fish. We approximate these dynamics by assuming that the entering migration block is allocated twice the effort as the block leaving the area (Table 1f). For additional days of fishing, only the migration block entering the area is fished (Table 1g).

Canadian Area 29

Area 29 is separated into two migration areas: below and above the Patullo Bridge at New Westminster, each containing one migration block. The harvest rate by gillnets in this area is very high and hydroacoustics data upstream of the fishery indicate that abundances are reduced to very low levels for almost 48 h after the commencement of a single-day fishery, which indicates almost complete removal of two migration blocks. Also, if a fishery is scheduled for 2 consecutive d, catches in the above Patullo Bridge area on the second day are reduced to approximately 8% of the catch below Patullo Bridge. Based on the harvest theory discussed so far, the predicted migration time to traverse each area should only be 1 d, in obvious contradiction to known travel time. To make the model results consistent with what is observed, we assume that no effort is directed on the fish entering the area (Table 1h). This results in the available fish in the upper area being virtually depleted on the second day, while the fishery in the lower area works on fresh fish.

Other Areas

Fisheries for gillnet gear only are scheduled in Canadian Areas 11, 12, and 20 and United States Areas 4B, 5, and 6C. The effort levels are low and the dynamics of exploitation are uncertain. Consequently, a simplistic model for these fisheries is used with the daily harvest rate related directly to effort

TABLE 4. Troll catches or harvest rates. For northern areas, catch is removed on an annual basis from the northern approach run prior to calculation of the timing abundance curve. These catches include troll and purse seine harvest.

	Comment	Early Stuart	Early miscellaneous	Horsefly/ late Stuart	Chilko/ Stellako	Birkenhead	Adams/ late runs
Catches							
Northern areas	Simulation 1	0	0	30 000	30 000	30 000	210 000
Northern areas	Simulation 2	0	0	60 000	60 000	60 000	420 000
Harvest (%)							
Area 127	Simulations 1 and 2	1.0	5.0	10.0	10.0	15.0	15.0
Area 124	Simulation 1	1.0	5.0	10.0	10.0	11.5	11.5
Aera 124	Simulation 2	1.0	2.5	5.0	5.0	6.0	6.0
Area 111	Simulation 1	0.6	1.0	2.0	3.0	4.0	4.0
Area 111	Simulation 2	0.6	2.0	3.0	5.0	7.5	7.5

TABLE 5. Summary of elementary harvest rates used in fisheries with purse-seine and gillnet gear. The elementary harvest rate (u) was assumed to be either constant or a simple function of fleet size over time.

	Comment	Harvest rate or relationship
Area 20	Simulation 1, all gears	$u = 0.85$
Area 20	Simulation 2, all gears	$u = 0.70$
Area 20	Gillnet fisheries only (equation (11))	$h = 0.0006 \times F$
Area 12	All area, all gears	$u = 0.48$
Area 12	Below Lewis Pt., all gears	$u = 0.66$
Area 12	q, gillnet fisheries only	$h = 0.0008 \times F$
Area 13	All area, all gears	$u = 0.28$
Area 16	All area, all gears	$u = 0.12$
Area 29B	Gillnet, summer-run and Birkenhead	$u = 0.925$
Area 29B	Late-run; inside run above gross escapement goal is completely harvested	
Area 29B	Inside troll; allocation removed from Gulf escapement of all stocks	
Area 29D	Gillnet, summer-run and Birkenhead	$u = 0.925$
Area 4B	q, gillnet, summer-run	$h = 0.002 \times F$
Area 4B	q, gillnet, late-run	$h = 0.001 \times F$
Area 7	All-gear relationship, summer-run	$u = 0.0928 \times \ln F - 0.1823$
Area 7	All-gear relationship, late-run	$u = 0.00088 \times F - 0.0443$
Area 7A	All-gear relationship, summer-run	$u = 0.2120 \times \ln F - 0.3602$
Area 7A	All-gear relationship, late-run	$u = 0.00377 \times F - 0.0405$
Areas 7 and 7A adjustment factor for Treaty Indian 16-h fishery		0.625
Areas 7 and 7A adjustment factor for non-Treaty 14-h fishery		0.681

and applied only to the fish entering the area ($k = r_{sf}$), i.e.,

(13) $$h_{ftr_{sf}} = q_f F_{ft}.$$

The west coast of Vancouver Island (WCVI) troll fishery is a complex fishery, with a very dynamic fishing effort. Fishermen routinely redirect fishing effort between species as prices and local salmon abundances fluctuate. The fishery is ideally modelled with all salmon species considered simultaneously (English et al. 1987). However, the fleet usually chooses to switch to sockeye when fishery regulations allow for their retention. Therefore, for sockeye, the application of equation (13) provides reasonable predictions of the catch and number of days of fishing required to achieve the domestic quota, although our approach is more simplistic than what occurs in the fishery.

Similarly, the inside area troll fishery is a complex, dynamic fishery that is difficult to model in conjunction with the other fishing areas. In the model, the inside troll quota is allocated from the total Area 29 catch, including the late-run sockeye catch, on an annual basis. We are currently evaluating procedures by which this complex fishery can be modelled in conjunction with the other fishing areas.

TABLE 6. Proportion (%) of catch by gear type. U.S. areas were not modelled.

	Purse seine	Gill net	Troll
Northern areas	25	0	75
Areas 111–127	0	0	100
Area 20	80	20[a]	0
Area 12	83	17[a]	0
Area 13	96	4	0
Area 16	75	25	0
Area 29B	0	—[b]	—[b]
Area 29D	0	100	0

[a]All-gear fishing days only; catch from gillnet-only fisheries is added to this catch.

[b]Area 29B: the inside troll allocation is removed from the total catch and the balance is taken by gillnets.

Inputs for 1990 Simulation for Preseason Planning

Modelling sessions begin with the collection of the relevant input information. Input information for the model comes from four sources: prediction information, historical

TABLE 7. Comparison of fishing plans (number of days or partial days by area) used in simulations 1 and 2. Codes are 1 for simulation 1 and 2 for simulation 2. Partial days of fishing for Treaty Indian (TI) and non-Treaty (NT) fishermen up to and including August 18 are simulated in U.S. Areas 7 and 7A only and full or traditional fisheries after that date.

Week end	Troll		Area 20		GN[a]		Areas 12 and 13		GN[b]		Area 16		Area 29		U.S. Area 4B		U.S. Areas 7 and 7A TI		U.S. Areas 7 and 7A NT		U.S. Areas 7 and 7A TI	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
July 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
July 14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
July 21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
July 28	0	0	0	0	0	0	0	0	2	2	0	0	1	1	4	4	1	0	1	0	0	0
Aug. 4	2	2	0	0	0	0	0	0	2	2	0	0	1	1	0	4	0	1	0	1	0	0
Aug. 11	7	7	0	1	0	0	1	1	2	2	1	1	2	1	3	3	1	1	1	1	0	0
Aug. 18	6	7	2	1	1	1	2	1	2	2	2	1	2[c]	2[c]	3	3	1	3	1	3	0	0
Aug. 25	0	6	2	1	1	2	2	2	2	2	2	2	2	2	2	2	1	3	1	3	1	0
Sept. 1	0	0	3	1	1	2	2	1	2	2	2	1	1	1	2	2	2	3	1	3	1	0
Sept. 8	0	0	2	0	1	0	1	1	2	0	0	0	1	0	0	0	0	2	0	0	0	0
Sept. 15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sept. 22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a]Area 20 gillnet fishery following combined purse-seine/gillnet fishery.
[b]Area 12 gillnet fishery following combined purse-seine/gillnet fishery.
[c]Split fishery, Monday and Wednesday, simulation 2 only.

data, policy data in the form of goals and objectives, and the fishing schedule itself. Prediction information can in fact be varied to test for sensitivity of the fishing plan to variability in these inputs. Predictions of total return by stock group are shown in Table 2 and are provided by the Canadian Department of Fisheries and Oceans (PSC 1991). Also provided by the Department of Fisheries and Oceans are predictions of timing by stock and the percentage of Fraser River sockeye expected to migrate through the northern marine areas and into Johnstone Strait or the so-called Johnstone Strait diversion (Mysak 1986). Diversion is a critical input that modifies the abundance of fish entering all fishing areas. Finally, the Department of Fisheries and Oceans provides forecasts of peak timing (Table 2) also during May (Blackbourn 1987).

Historical information is used for inputs on run distributions, routing, migration speeds, harvest rates, and fleet dynamics (Table 2–6). Run reconstructions and racial analysis are the source of these data (Gable and Cox-Rogers 1993).

Policy information in the form of goals and objectives is an important input to the modelling process. The Fraser River Panel of the Pacific Salmon Commission is closely involved in the guidance of the preseason planning process. The Department of Fisheries and Oceans provides gross escapement goals by stock grouping (see Table 9). This includes net spawning escapement plus the projected catch in the Fraser River Indian Food Fishery (e.g., Macdonald 1990). Under the terms of the Pacific Salmon Treaty, the United States catch entitlement is a maximum of 7 000 000 sockeye for the period 1989–92 of which the United States elected to catch 2 200 000 in 1990 (PSC 1991). While the domestic allocation goals are subordinate to the international allocations, they are extremely important to the user groups within the two countries and attempts are made to accommodate them (PSC 1991). These domestic goals include individual allocations to the gear sectors in Canada and to the Treaty Indian and Non-Treaty user groups in the United States (see Table 10). The sources of this information are the respective national (and from the United States, tribal) agencies. Individual allocations to gear sectors in the United States (Non-Treaty fishery), while important, were not modelled, as harvest rate inputs were not considered sensitive enough to resolve such allocations accurately. Fisheries would be adjusted in-season as necessary to accommodate these goals. In 1990, because of chinook salmon (*Oncorhynchus tshawytscha*) conservation concerns, fisheries on late-run stocks by gillnets in Canadian Area 29 were limited such that no fishing was to be scheduled in that area after September 5. Because the marine area harvest by gillnetters of sockeye in general and late-run sockeye in particular would provide only minor contribution to the gillnet allocation (see Table 10), the major portion of the gillnet allocation was to be taken from summer-run sockeye in Area 29. This required limitation of all-gear fisheries early in the season in marine fishing areas in both Canada and the United States. The United States catch of summer-run sockeye was to be limited to 586 000 through the reduction of early-season fisheries.

The final type of information for the model is the fishing plan itself (Table 7). The actual historical levels of fishing by week should be used as a starting point from which the modelling process develops. The ultimate fishing plan is developed by an iterative process, guided by goals and objectives.

Results

The simulations that are presented below were chosen to show the dramatic effects of variation in the Johnstone Strait diversion on a fishing plan; however, variation in timing and run size will also result in considerable variation to fishing plans, both preseason and in-season, in order to achieve the goals. The first simulation was made using a season average Johnstone Strait diversion of 24% and the second simulation was made using a season average of 55%. The fishing plan in each case was optimized to achieve the goals in order of importance: conservation (escapement goals), international allocation, and domestic allocation

TABLE 8. Weekly catch summary by area, simulations 1 and 2. The WCVI troll catch does not include annual modelled catches for the northern area troll. Area 29 catches include troll and gillnet but do not include Adams River or late-run stocks, the latter of which are modelled as an annual terminal catch.

Week end	WCVI troll	Area 20	Areas 12 and 13	Area 16	Area 29	Area 4B	Areas 7 and 7A		
							Treaty Indian	non-Treaty	Total
				Simulation 1					
July 7	0	0	0	0	0	0	0	0	0
July 14	0	0	0	0	0	0	0	0	0
July 21	0	0	0	0	0	0	0	0	0
July 28	0	0	7 135	0	100 056	44 168	68 057	92 661	312 077
Aug. 4	155 000	0	11 037	0	225 044	0	0	0	391 081
Aug. 11	777 771	0	243 924	17 701	536 048	45 350	140 123	164 784	1 925 701
Aug. 18	748 133	662 148	606 830	37 881	367 114	27 732	76 476	137 529	2 663 843
Aug. 25	0	875 764	649 152	53 065	105 853	22 174	284 022	366 850	2 356 880
Sept. 1	0	795 168	291 056	37 904	17 920	18 172	412 919	351 284	1 924 423
Sept. 8	0	125 672	38 252	0	11 336	0	0	0	175 260
Sept. 15	0	0	0	0	0	0	0	0	0
Sept. 22	0	0	0	0	0	0	0	0	0
Total	1 680 904	2 458 752	1 847 386	146 551	1 363 371	157 596	981 597	1 113 108	9 749 265
				Simulation 2					
July 7	0	0	0	0	0	0	0	0	0
July 14	0	0	0	0	0	0	0	0	0
July 21	0	0	0	0	0	8 070	0	0	8 070
July 28	0	0	12 736	0	64 361	19 668	0	0	96 765
Aug. 4	112 962	0	25 101	0	167 810	38 088	70 921	89 189	504 071
Aug. 11	549 704	135 789	619 001	42 921	280 432	28 823	88 919	91 133	1 836 722
Aug. 18	528 784	217 576	1 040 670	62 354	337 554	21 354	184 828	88 753	2 481 873
Aug. 25	210 314	307 593	1 458 951	142 324	124 741	14 771	276 417	497 307	3 032 418
Sept. 1	0	171 066	489 240	56 821	36 474	9 601	251 072	330 182	1 344 456
Sept. 8	0	0	80 762	0	0	0	70 265	65 180	216 207
Sept. 15	0	0	0	0	0	0	0	0	0
Sept. 22	0	0	0	0	0	0	0	0	0
Total	1 404 764	832 024	3 726 461	304 420	1 011 372	140 375	942 422	1 161 744	9 520 582

among gear types and user groups within countries. Weekly catches by area are shown in Table 8 and the annual catch by area, actual and target harvest rates, and actual and target escapement by stock are shown in Table 9. These results represent an entire management season for a group of salmon stocks and summarize catch and escapement data that would be collected during the fishing season. Table 10 shows the comparison between international and domestic catch goals and realized catches.

The troll fishery, which opens on August 3, proceeds very quickly and is closed with the troll allocation taken by August 17. Considerable fishing in Area 20 for purse seines and gillnets is scheduled after August 11. The fishing times in United States waters early in the season were reduced, with a closed period scheduled for the week ending August 4. The United States catch limitation on summer-run sockeye was achieved following the fishing plan described in Table 7.

In the second simulation where the effects of high Johnstone Strait diversion are simulated, the abundance of sockeye migrating through the south approach is reduced to the point that extensions in the troll fishing season (Table 7) are necessary in order for that gear to reach the allocation. In addition, the troll catch in the Queen Charlotte Islands has been increased in expectation that the northerly landfall of sockeye onto the coast would result in a shift in troll effort further north. A reduced fishing schedule in Area 20 is necessary in order to allow sufficient abundance of fish for harvest in United States waters. The amount of fishing time in United States waters was also increased, particularly during the period of late-run migration in order to harvest the quota from the lower daily abundance in United States waters. Although fishing times in Area 20 were reduced substantially, Canadian purse seiners have little difficulty in reaching their allocation; in fact, fishing times in Areas 12–16 must be reduced in order to allow for sufficient escapement into Area 29 for conservation and for harvest by gillnet and inside troll fishermen. The very large catches in Areas 12 and 13 are due in part to the very high daily harvest rate as well as the length of the effective fishing area, which is much larger than Area 20. Fishing times in Area 29 have also been reduced to allow for escapement goals to be met.

The gross escapement by stock is shown in Table 9. The timing of Horsefly/Late Stuart and Chilko/Stellako stocks is overlapped such that the gross escapement goal of Horsefly conflicts with the harvest requirements of Chilko sockeye. A decision must be made as to which of these two conflicting goals is more important. We finally chose to manage

TABLE 9. Summary by stock of annual catch by area, model, and target escapements and actual and target harvest rates for simulations 1 and 2. MIFF sport category is for the marine area Indian food fishery and sport fishery.

	Summer-run stocks				Late-run stocks		
	Early Stuart	Early miscellaneous	Horsefly/ late Stuart	Chilko/ Stellako	Birkenhead	Adams/ late	Total
			Simulation 1				
Northern area	0	0	30 000	30 000	30 000	210 000	300 000
Outside troll	0	11 525	61 660	74 392	154 405	1 378 922	1 680 904
Area 20	0	16 655	37 705	57 626	171 476	2 175 290	2 458 752
Johnstone Strait	1	18 587	63 905	90 315	140 734	1 533 844	1 847 386
Area 16	0	2 638	9 302	11 572	12 265	110 774	146 551
Area 29	1 551	254 527	577 219	406 952	123 122	384 588	1 747 959
United States	339	126 281	267 081	192 384	161 896	1 504 320	2 252 301
MIFF/sport	0	2 000	15 000	15 000	10 000	44 000	86 000
Test fishery	4 000	6 000	13 000	10 000	7 000	35 000	75 000
Total catch	5 891	438 213	1 074 872	888 241	810 898	7 376 738	10 594 853
Escapement							
Model	119 109	492 787	925 128	561 759	314 102	3 392 262	5 805 147
Target	125 000	507 000	981 000	459 000	316 000	3 392 000	5 777 000
Annual harvest rates (%)							
Model	4.7	47.1	53.7	61.3	72.1	68.5	64.6
Target	2.4	45.5	50.9	68.3	71.9	68.5	64.8
			Simulation 2				
Northern area	0	0	60 000	60 000	60 000	420 000	600 000
Outside troll	0	6 858	54 414	54 769	104 661	1 181 062	1 401 764
Area 20	0	22 973	73 974	63 389	63 931	607 757	832 024
Johnstone Strait	1	40 476	243 466	202 256	276 308	2 963 954	3 726 461
Area 16	0	5 677	30 878	23 973	24 989	218 903	304 420
Area 29	1 504	201 834	413 611	282 103	112 320	388 381	1 399 753
United States	171	124 146	253 728	191 421	157 314	1 517 761	2 244 541
MIFF/sport	0	2 000	15 000	15 000	10 000	44 000	86 000
Test fishery	4 000	6 000	13 000	10 000	7 000	35 000	75 000
Total catch	5 676	409 964	1 158 071	902 911	816 523	7 376 818	10 669 963
Escapement							
Model	119 324	521 036	841 929	547 089	308 477	3 392 182	5 730 037
Target	125 000	507 000	981 000	459 000	316 000	3 392 000	5 777 000
Annual harvest rates (%)							
Model	4.5	44.0	57.9	62.3	72.6	68.5	64.1
Target	2.4	45.5	50.9	68.3	71.9	68.5	64.8

the summer-run stocks as a complex, and tried to attain a combined stock goal. We were able to model international and domestic catches within 5% of the allocation required.

Discussion

The model that we have described in this paper has been used to determine how the spawning escapement and international allocation goals can be achieved through the development of preseason fishing plans on Fraser River sockeye. Its results are generally accepted in this process, and another comodel is used for pink salmon (*Oncorhynchus gorbuscha*) stocks originating in rivers south of Cape Caution. These models are also used in guiding the domestic planning process for fisheries on Fraser sockeye and pink salmon in both Canada and the United States.

The fishing plans determined by using the model are wholly dependent on the reliability of the input data. The in-season realities of timing, run size, diversion rate, and travel speeds are often very different from the preseason predictions used for model inputs to develop fishery plans (PSC 1991). Consequently, the model results for the preseason planning process serve only as a starting point for in-season management. If post-season estimates of abundance, timing, and diversion rate are used in hindsight with the actual in-season fishing schedule, the catches and escapements indicated by the model agree relatively well with actual experience. However, small-scale predictions of daily catch by a fishery are not always precise because daily abundances of fish, while on average normally distributed, tend to be more variable in reality. Users of this and similar models must be aware of this tendency.

The decision to highlight the effects of variation in Johnstone Strait diversion on the planning process in the results was not entirely arbitrary, although the sensitivity of fishing plans to other inputs also could have been demon-

TABLE 10. Comparison between target and modelled catches and domestic allocations.

Category	Target	Simulation 1	Simulation 2
Total run	16 400 000	16 400 000	16 400 000
Gross escapement	5 777 000	5 805 147	5 678 752
Commercial catch	10 462 000	10 433 853	10 508 963
U.S. catch	2 200 000	2 252 301	2 244 541
Treaty Indian	1 100 000	1 139 193	1 082 797
Non-Treaty	1 100 000	1 113 108	1 161 744
Canadian catch	8 262 000	8 181 552	8 264 422
Other catch[a]	161 000	161 000	161 000
Canadian domestic allocations (%)			
Purse seine	45.5	45.2	46.2
Gill net	28.0	27.7	27.2
Outside troll	22.7	23.3	22.8
Inside troll	3.8	3.8	3.8

[a]Test fishery catch, marine area Indian food fishery and sport fishery.

strated. In fact, the two simulations provided as examples were chosen from those investigated during the planning process. Initially in this process, simulations were investigated using low levels of diversion. However, in early June 1990, high sea-surface temperatures in the North Pacific were evident, suggesting a higher than normal "landfall" of Fraser River sockeye onto the coast, and plans were modelled, and subsequently adopted, taking into account the expected high Johnstone Strait diversion. The preseason management plans that were adopted for Panel Area waters (PSC 1991) developed from results of the high diversion scenario presented here. Under the scenario of high diversion, although the total run size does not change, the relative abundances of sockeye entering all fishing areas are altered. Since the level of exploitation varies among fishing areas, the adjustment of fishing times in all areas is essential as Johnstone Strait diversion changes. The high diversion did not materialize in 1990 and actual fisheries were substantially delayed because the timing of the summer-run stocks was 2 wk later than anticipated (PSC 1991).

Sprout and Kadowaki (1987) have reviewed the importance of explicitly stating objectives in managing salmon stocks. We agree that policy information in the form of goals and objectives is essential to guide the planning process and, as already mentioned, the sources of this information are the various fisheries agencies. The Fraser River Panel of the Pacific Salmon Commission (PSC 1990b) is involved with implementing many aspects of policy to guide both the preseason and in-season planning process. The staff of the Pacific Salmon Commission, in consultation with the Fraser River Panel Technical Committee, is given the task of developing the preseason fishing options in Panel Area waters for review, revision, and subsequent approval by the Fraser River Panel. Canadian members of the Technical Committee coordinate the development of fishing scenarios of non-Panel Area waters in Canada for the modelling process. Our experience has been that the results of planning sessions during the early development of the model clearly indicate the need for policy direction. When such direction is absent, participants in the modelling process tend to be frustrated by the lack of agreement and progress in the sessions. Modelling sessions have affected harvest strategies and have prompted the formulation of policy needed in the management of Fraser River sockeye fisheries. In these situations, the model has shaped negotiations on whether certain user groups should obtain catch before other users gain access to the resource. The participants in the modelling sessions have debated whether this is proper use of the model.

Goals need to be ranked in order of importance, a process that sometimes requires political solutions. These typically include escapement goals by stock, catch-sharing arrangements, access by certain user groups to particular stocks, preservation of historical patterns, and the need to fish throughout the season. Conservation concerns are typically ranked first, followed by international allocation goals and finally domestic allocation goals. If the attainment of the escapement goal for one stock conflicts with the desired catch goal of another stock, the managers of the resource must determine the relative importance of the conflicting goals. In many situations, the objective to preserve historical fishing patterns is not achievable, as stock rebuilding and new international and domestic allocation goals are now considered more important. In these situations, the model results are particularly useful in forcing participants in the preseason planning process to focus on issues that would otherwise not be addressed until fisheries start, and then perhaps too late for thoughtful resolution.

While we have attempted to develop a model rigorously based on the theory of fish movement and harvest, in the case of Canadian Area 29, we have been less successful in resolving the instantaneous nature of the fishery with the daily time step. As indicated previously, to make the model results consistent with what is observed, we assume that no effort is directed on the fish entering the area. In this situation, we believe that modelling the known result is more important than being entirely consistent with theory on fish movement and harvest, as the principal purpose of the model is for assessing fishery options on conservation and harvest goals.

In consideration of stock reconstruction, our model can also run "backwards" to obtain estimates of harvest rates for catch accumulated over any time period. However, we must specify a migration sequence of fisheries common for all stocks because we allow members of a stock to be present in more than one fishery during the period over which catch is accumulated. If, as assumed by Starr and Hilborn (1988), the members are restricted to a single fishery during this period, then the migration sequence is immaterial.

The quantification of fish movement and exploitation applied in this model has proved valuable for in-season management as well as for preseason planning. Forwards and backwards versions of the model can be used to estimate the theoretical cumulative harvest rate for a migrating block over the full range of fisheries. These harvest rates are then applied to cumulative catch curves by stocks that have been estimated using direct racial analysis of catch. This procedure has been useful for determining escapements of late-run stocks of both Fraser sockeye (such as Adams River and Lower Shuswap River stocks) and pink salmon that delay in the Gulf of Georgia for 2–4 wk before migrating upstream. The timely estimation of terminal escapement is important for fine-tuning the management of these stocks (Woodey 1987). The theory of fish movement and exploitation used in this model has also stimulated the development of alternative run-size estimation models for in-season use (PSC 1990b). In the future, we believe that in-season models could be developed using inputs from the preseason model, after rigorous testing of the assumptions inherent in the present model.

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Appendix: Notation

Indices

f	fishery
j	fishery referenced in sequence
k, l	migration block of fish available to a fishery
m	time period consisting of more than 1 d (e.g., week)
s	stock
t	time (days)

Variables

a_f	regression coefficient for fishery f
A	temporary variable holding the population to be updated
b_f	regression coefficient for fishery f
C_{ft}	catch in fishery f during the tth day
C_{fts}	catch in fishery f of stock s during the tth day
C_{ftsk}	catch in fishery f of stock s from the kth migration block during the tth day
D_s	duration or spread (days) of stock s
E_{ft}	escapement from fishery f during the tth day
E_{st}	escapement of stock s during the tth day
F_{ft}	size of the fishing fleet in fishery f during the tth day
h_{ft}	harvest rate of fishery f during the tth day
h_{ftk}	harvest rate of fishery f on the kth migration block during the tth day
J	last fishery through which fish migrate common to all stocks
J_s	last fishery through which stock s migrates prior to escapement
n	number of stocks
N_C	fish that are at risk to fishing
N_E	fish that are not at risk to fishing
N_s	total return of stock s
N_{st}	available population representing the run (planning model) or escapement (stock reconstruction) entering the first fishery of stock s during the tth day at the commencement of the model
O_j	fishery identifier as any stock migrates through the jth area
O_{sj}	fishery identifier as stock s migrates through the jth area
p_{ftk}	weight given to fishing intensity in fishery f on the kth migration block during the tth day scaled to 1
q_f	catchability coefficient of fishery f
r_{sf}	residence (days) of stock s in fishery f
T	number of days in the season
$t_{\max_s}$	time (days) at peak run size of stock s (mean of normal distribution)

u_{ft} elementary harvest rate of fish entering or leaving fishery f during the tth day

w_{sf} travel time (days) by stock s to travel from entry in the first fishery to exit in fishery f

x constant that controls the harvest contrast of fish entering or leaving a fishery and of fish fully resident in the fishery

Z_α standard normal value at confidence level $1 - \alpha$

σ_s deviation in days (normal distribution) of stock s.

When Did Acid-Sensitive Adirondack Lakes (New York, USA) Begin to Acidify and Are They Still Acidifying?

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A transfer function was used to reconstruct pH values of 20 low-alkalinity Adirondack Park lakes based on the species composition of scaled chrysophytes (Chrysophyceae, Synurophyceae) in stratigraphic intervals from ^{210}Pb-dated sediment cores. Approximately 80% of the lakes acidified since preindustrial times. Four categories of lake response to acidic deposition were identified: (i) lakes that showed little or no evidence of acidification since preindustrial times, (ii) lakes with preindustrial pH values between 5 and 6 that began to acidify ca. 1900, (iii) "naturally" acidic lakes that acidified even further ca. 1900, and (iv) lakes with preindustrial pH values around 6 that acidified ca. 1930–50. Lakes that acidified ca. 1900 were generally smaller, higher elevation lakes with lower preindustrial pH values than lakes in category i or iv. These patterns are consistent with the acidic deposition hypothesis of recent lake acidification. Our results indicated that "critical" sulfate deposition loads for Adirondack lakes that started to acidify ca. 1900 and ca. 1950 are between 5–10 and 20–25 $kg \cdot ha^{-1} \cdot yr^{-1}$, respectively. Post-1970 trends in lake water pH have been small and variable, suggesting that low-alkalinity Adirondack lakes have been relatively unresponsive to the post-1970 declines in sulfate deposition.

Une fonction de transfert a été utilisée pour reconstituer les valeurs du pH de 20 lacs de faible alcalinité du Parc des Adirondacks en s'appuyant sur la composition en espèces d'une échelle de chrysophytes (Chrysophyceae, Synurophyceae) dans des intervalles stratigraphiques à partir de carottes de sédiments datées au ^{210}Pb. Environ 80 % des lacs se sont acidifiés depuis l'ère pré-industrielle. Quatre catégories de réponse des lacs aux dépôts acides ont été dégagées : i) les lacs où on observe peu ou pas de signe d'acidification depuis l'ère pré-industrielle, ii) les lacs qui possédaient un pH pré-industriel variant entre 5 et 6 et qui ont commencé à s'acidifier autour de 1900, iii) les lacs « naturellement » acides qui se sont acidifiés encore davantage autour de 1900 et iv) les lacs dont le pH pré-industriel était approximativement de 6 et qui se sont acidifiés entre 1930 et 1950. Les lacs qui se sont acidifiés autour de 1900 étaient généralement plus petits, situés à une altitude plus élevée et leur pH pré-industriel était plus faible que les lacs des catégories i ou iv. Ces observations sont en accord avec l'hypothèse du dépôt acide de l'acidification récente des lacs. Nos résultats indiquent que les charges «critiques » des dépôts de sulfate pour les lacs des Adirondacks qui ont commencé à s'acidifier autour de 1900 et de 1950 sont respectivement comprises entre 5–10 et 20–25 $kg \cdot ha^{-1} \cdot an^{-1}$. Les tendances observées après 1970 en ce qui concerne le pH de l'eau des lacs sont de faible amplitude et variables, ce qui semble indiquer que les lacs des Adirondacks de faible alcalinité ont relativement peu répondu à la diminution des dépôts de sulfate après 1970.

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Lake acidification is a major international problem and the focus of much scientific research. Several hypotheses have been put forward to explain regional lake acidification, including natural long-term processes (e.g., Pennington 1984), watershed disturbances and soil and vegetation changes (e.g., Rosenqvist 1978, 1980; Krug and Frink 1983; Krug 1989; Dobson et al. 1990), and acidic deposition (e.g., Turner et al. 1990). Many lines of evidence including studies that have examined the current chemical status and spatial distribution of lakes (e.g., Schofield 1982; Sullivan 1990), whole-lake experimental acidification studies (e.g., Schindler et al. 1991; Wright and Hauhs 1991), monitoring and resurvey data (e.g., Asbury et al. 1989), and paleolimnological investigations (e.g., Charles et al. 1989; Charles 1990; Battarbee et al. 1990) all support the acidic deposition theory as the major cause of recent regional lake acidification in North America and Europe. However, because of the lack of historical data, little information is available on the timing and trajectories of lake acidification.

In the absence of long-term water chemistry data, paleoecological techniques provide the most reliable way to estimate past changes in lake water pH (Charles et al. 1989). Most of these analyses have been based on sedimentary diatom assemblages, but recently, chrysophyte assemblages have been

used increasingly to reconstruct historical pH profiles from ^{210}Pb-dated sediment cores (e.g., Smol et al. 1984a, 1984b; Cumming et al. 1992b; Dixit et al. 1992). Scaled chrysophytes (Synurophyceae and Chrysophyceae, sensu Anderson 1987) are an important component of the algal flora in most freshwater lakes (Kristiansen 1986) and are characterized by overlapping siliceous scales and bristles on which the taxonomy of these planktonic organisms is based. The distribution of chrysophyte taxa has been shown to be closely related to lake water pH (e.g., Siver and Hamer 1989). For example, in the Adirondack region, quantitative estimates of the pH optima (abundance-weighted mean pH of occurrence) and tolerance (abundance-weighted standard deviation) of chrysophyte taxa to pH have been estimated based on both live (e.g., Siver and Hamer 1989) and sedimentary samples (e.g., Cumming et al. 1992a). Transfer functions have been developed that can infer pH with a high degree of confidence from the relative abundance of chrysophyte species in a sediment sample (Cumming and Smol 1993).

The use of both diatoms and chrysophytes in paleoecological studies is advantageous because they provide two independent techniques to reconstruct past lake water conditions (e.g., Cumming et al. 1992b; Dixit et al. 1992). However, enumeration of scaled chrysophytes is much quicker than diatoms because there are fewer taxa and their taxonomy is more straightforward. A number of studies suggest that scaled chrysophytes are more sensitive indicators of changes in lake water pH than diatoms. For example, the response of scaled chrysophytes almost always predates diatom changes in investigations of lake acidification and recovery (e.g., Gibson et al. 1987; Dixit et al. 1990; Dixit et al. 1992). In addition, a number of paleolimnological studies suggest that scaled chrysophytes may respond to episodic acidification to a greater extent than diatoms (e.g., Gibson et al. 1987; Cumming et al. 1992b). Because chrysophytes are relatively easy to identify and are sensitive to changes in lake water pH, they are a valuable tool for assessing the history of lake acidification, although the statistical accuracy of pH-inference models is not as high as that of diatom-based models (e.g., Dixit et al. 1993).

Recent refinements in sediment coring and sectioning procedures (Glew 1988) and advances in pH-inference models make it possible to detect recent changes in lake water chemistry from the changes in the composition of scaled chrysophytes. Several studies have now been completed that show good agreement between trends in chrysophyte-based pH reconstructions and long-term water chemistry measurements (e.g., Dixit et al. 1989, 1992; Cumming 1991; Sullivan et al. 1992).

The PIRLA-II (Paleoecological Investigation of Recent Lake Acidification II, Charles and Smol 1990) project addressed two major issues related to lake acidification. The first issue assessed the extent and magnitude of recent lake acidification in the Adirondack region. Based on a set of statistically chosen lakes from a predefined target population of 675 relatively small (surface areas between 4 and 200 ha and watershed <2000 ha), undisturbed, low-alkalinity lakes, Cumming et al. (1992b) estimated that 25–35% (169–236 lakes) of these lakes had acidified since the 1800s, and almost all (>80%) lakes with preindustrial pH values <6 acidified. The lakes that acidified were mainly located in the southwestern Adirondacks, a region that receives the highest amount of acidic deposition and is geologically susceptible to the effects of acidic precipitation (Sullivan et al. 1990b; Cumming et al. 1992b).

Previous paleoecological studies have shown that many currently acidic lakes in the Adirondack region have acidified since preindustrial times (e.g., Del Prete and Schofield 1981; Charles and Norton 1986; Davis et al. 1988; Charles et al. 1990). Acidification occurred primarily between 1920 and 1960 and coincided with increases in the combustion of by-products such as Pb, Cu, V, Zn, S, PAH, and coal and oil soot (Charles et al. 1990). However, quantitative pH trajectories have been completed for only 12 lakes (Davis et al. 1988; Charles et al. 1990). Due to the paucity of long-term historical measurements and paleoecological studies (Driscoll et al. 1991), the second major issue addressed by the PIRLA-II project and the focus of this paper was to determine when low-alkalinity Adirondack lakes acidified and if these lakes are now recovering since the post-1970 declines in sulfate deposition.

To this end, we use paleoecological techniques to reconstruct the post-1850 temporal trends in pH from 20 acid-sensitive Adirondack region lakes (pH values generally <6) to determine the timing, rate, and magnitude of acidification. In addition, we focused on the post-1970 pH trends in these lakes to determine if they are currently in "steady state", continuing to acidify, or increasing in lake water pH. The resultant pH trends were assessed qualitatively and quantitatively from the stratigraphic changes in the relative abundance of chrysophyte taxa in the top 20–30 cm from ^{210}Pb-dated sediment cores. This information is relevant to limnologists, water managers, and policy makers interested in the effects of anthropogenic pollution on lakes.

Methods

Study Sites and Sample Collection

The Adirondack Park is a large (~2 600 000 ha, Colquhoun et al. 1984), forested mountainous area in northern New York State (USA). The political boundary of the Park generally corresponds to the Adirondack ecological zone, which is broadly defined as the area lying above the 305-m elevation contour surrounding the Adirondack upland and mountain area, and consists mainly of igneous and metamorphic rocks that are geologically similar to the Laurentian Shield of Canada (Driscoll et al. 1991). Additional information on the climate, geology, soils, and vegetation can be found in Driscoll et al. (1991).

Within the Adirondack ecological zone, there are 2796 lakes and ponds that are greater than 0.2 ha in surface area (Adirondack Lake Survey data in Driscoll et al. 1991). Atmospheric precipitation is highly acidic (pH < 4.5), and inputs of acidic materials are now widely believed to be responsible for the recent acidification of many lakes. Elevated monomeric Al concentrations are toxic to many aquatic organisms (Baker and Christensen 1991) and are positively correlated with acidity in Adirondack lakes (Driscoll et al. 1991). Estimates of the number of currently acidic lakes (ANC < 0 $\mu eq \cdot L^{-1}$) in the Adirondack region range from ~11% (for a population of lakes with surface areas >4 ha, Brakke et al. 1988) to 26% (lakes with surface area >0.5 ha, Kretser et al. 1989). Furthermore, Eshleman (1988) has estimated that 35% of Adirondack lakes could experience acidic conditions during the spring, following the inputs of acidic waters from snowmelt.

TABLE 1. Selected morphometric and physical characteristics for the 23 study lakes. For comparative purposes, the lakes are arranged according to their chrysophyte-inferred acidification trajectories in the following order (i) lakes that showed little or no evidence of lake acidification since preindustrial times or lakes that underwent limited acidification and have subsequently recovered, (ii) lakes with preindustrial pH values between 5.5 and 6 that began to acidify ca. 1900, (iii) lakes that were "naturally" acidic but acidified further ca. 1900, (iv) lakes with preindustrial pH values between 5.5 and 6.0 that acidified ca. 1930–50, and (v) lakes that were eliminated due to preservation problems and/or mixed ^{210}Pb dating profiles. Data for watershed area (WA), lake area (LA), and water retention time (RT) were primarily obtained from the Direct/Delayed Response Project (DDRP) database (Church et al. 1989). Other sources for WA, LA, and RT include the Adirondack Lake Survey Corporation (Raven Pond, Twin Pond (east), Evergreen Lake, and Bellows Lake), the Eastern Lake Survey (Rock Pond, Squash Pond, Kanciruk et al. 1986), the Lake Acidification Mitigation Project (Woods Lake (BM), Staubitz and Zarriello 1989; Little Simon Pond, McAvoy and Driscoll 1989), and the Integrated Lake-Watershed Acidification Study (Goldstein et al. 1987). Additional abbreviations: ELS, Eastern Lake Survey; BM = Big Moose region; SL = Silver Lake Wilderness Area.

Lake	ELS identification	Hydrologic type[a]	Elevation (m)	Maximum depth (m)	LA (ha)	WA (ha)[b]	WA/LA	RT (yr)
Woodhull Lake	1A1-066	D	572	27	399	1383	4	—
John Pond	1A1-039	DO	546	8	6	38	7	—
No Name Pond (Mud Pond)	1A2-048	DO	485	11	5	41	9	0.3
Long Pond	1A3-046	D	565	13	14	134	10	0.8
Little Simon Pond	—	D	550	33	63	764	12	1.2
Fourth Lake Bisby	1A1-020	D	609	9	23	266	12	0.5
Constable Pond	1A1-017	D	582	4	19	888	48	0.1
Trout Lake	1A2-054	D	651	9	17	220	13	0.2
Bellows Lake	1A2-031	D	546	6	12	1550	129	0.2
Gull Lake (South)	1A1-073	DO	594	4	10	108	10	0.3
Hitchcock Lake	1A1-057	DO	567	14	15	125	9	0.3
Raven Lake	1A1-016	D	587	24	47	547	12	0.9
Evergreen Lake	1A1-004	D	613	18	18	52	3	2.0
Squash Lake	1A1-111	DO	650	7	2	82	41	0.1
Hawk Pond	1A1-003	D	645	10	13	80	6	1.5
North Branch Lake	1A2-042	D	575	8	8	172	22	0.2
Twin Ponds (East)	1A2-018	DO	413	13	5	33	7	1.3
Big Moose Lake	1A1-103	D	557	20	520	9555	18	0.2
Woods Lake (BM)	—	D	601	12	23	184	8	0.5
South Lake	1A3-065	D	615	18	196	1375	7	0.7
St. John Lake	1A2-002	D	673	11	13	138	11	0.9
Rock Pond	1A3-066	D	570	3	13	96	7	0.3
Woods Lake (SL)	1A2-045	D	418	12	26	149	6	1.7

[a]D = drainage; DO = drainage (outlet only).
[b]Does not include LA.

Lakes within the Adirondack Park have been intensely studied for the effects of recent lake acidification (Sullivan 1990; Driscoll et al. 1991). These studies include dynamic geochemical model estimates (e.g., Cosby et al. 1985a, 1985b) of past (Wright et al. 1986; Sullivan et al. 1990a) and future change (Church et al. 1989; Hunsaker and Graham 1991), historical and recent water chemistry data (e.g., Asbury et al. 1989; Driscoll et al. 1991; Driscoll and Van Dreason 1993), and paleolimnological studies (see the introduction).

In general, our study lakes can be classified as relatively undisturbed, low-alkalinity to highly acidic lakes, with short water residence times from the southwestern region of the Park (Tables 1 and 2; Fig. 1). Ratios of watershed area to lake area range from 3 to 129. The majority of these lakes should have acidified since preindustrial times (Cumming et al. 1992b) and are therefore of considerable interest with regard to their acidification patterns and possible recoveries since the post-1970 decline in sulfate deposition.

With the exception of cores from North Branch Lake and one of the four cores from Big Moose Lake, fieldwork was carried out in July and August of 1988. The cores from North Branch Lake were taken in May 1988 whereas one of the cores from Big Moose Lake was taken in July 1985 (for coring details from this site, see Charles et al. 1991). To show that post-1970 trends are reproducible, replicate cores were removed from Big Moose Lake in August 1988. PIRLA-I coring and sectioning protocols were followed (Charles and Whitehead 1986a, 1986b), with a few minor modifications (Smol et al. 1989). Briefly, a modified Kajak–Brinkhurst gravity corer (Brinkhurst et al. 1969), equipped with a modified trigger mechanism (Glew 1989) and a 50-cm-long Plexiglas core tube (internal diameter 6.5 cm), was used to obtain sediment cores from the deepest basin of the study lakes. Sediment cores can be retrieved with minimal compaction and/or sediment smearing using proper coring procedures (Cumming et al. 1993).

Using an extrusion system designed to section sediment cores at close intervals (Glew 1988), the cores were sectioned, on the lake shore, at 0.25-cm intervals for the top 5 cm, at 0.5-cm intervals for 5–10 cm, and at 1-cm intervals for the rest of the core. Bathymetric maps showing the approximate location of the coring sites, along with other notes on the physical characteristics of the sediment cores, are in Cumming et al. (1989). Quality assurance design and implementation are documented in Charles and Whitehead (1986b), Cumming et al. (1990), and Charles et al. (1991).

TABLE 2. Selected mean lake water chemistry and watershed disturbance data for the 23 study lakes, arranged in the same order as in Table 1. Water chemistry data are arithmetic averages taken from Eastern Lake Survey (ELS-I) (Kanciruk et al. 1986) and ELS-II (if available). Waterchemistry values for Woods Lake (BM) and Little Simon Pond are not given because these lakes have been the subject of large liming projects (see text for details). Data for cabin count, % wetlands in watershed, and % conifers in watershed were primarily obtained from the Direct/Delayed Response Project (DDRP) database (Church et al. 1989). The other source for cabin count, % wetlands in watershed, and % conifers in watershed was the Adirondack Lake Survey Corporation (Raven Pond, Twin Ponds (east), Evergreen Lake, and Bellows Lake). Abbreviations: ANC = gran acid-neutralizing capacity; AI_l = labile monomeric aluminum; DOC = dissolved organic carbon.

Lake	pH (aerated)	ANC ($\mu eq \cdot L^{-1}$)	AI_l (μM)	Total AI ($\mu g \cdot L^{-1}$)	DOC (μM)	Cabin count	Liming[a]	% wetlands in watershed	% conifers in watershed
Woodhull Lake	5.5	4	1.4	82	155	20	0	2	2
John Pond	5.3	2	3.0	122	101	0	0	0	0
No Name Pond (Mud Pond)	5.1	−4	1.9	141	446	0	0	11	43
Long Pond	5.8	27	0.3	32	247	0	0	0	6
Little Simon Pond	—	—	—	—	—	1	1	—	—
Fourth Lake Bisby	5.9	17	3.1	203	347	0	2	10	5
Constable Pond	4.8	−7	7.2	420	354	0	0	1	10
Trout Lake	4.8	−9	5.4	314	400	0	0	5	8
Bellows Lake	5.3	3	1.8	168	78	0	0	3	0
Gull Lake (South)	4.6	−15	10.0	425	278	0	0	0	27
Hitchcock Lake	4.9	−9	7.5	416	498	0	0	0	19
Raven Lake	4.6	−8	10.9	516	171	2	0	0	20
Evergreen Lake	4.7	−11	6.1	273	88	4	0	3	10
Squash Lake	4.6	−18	5.7	685	610	0	0	—	—
Hawk Pond	4.7	−14	0.0	425	278	0	0	0	33
North Branch Lake	5.5	7	3.4	293	399	2	1	1	6
Twin Ponds (east)	4.8	−13	2.6	308	435	0	0	0	20
Big Moose Lake	5.4	5	1.4	258	196	151	1	—	—
Woods Lake (BM)	—	—	—	—	—	1	1	—	—
South Lake	5.3	4	1.8	158	143	26	0	2	0
St. John Lake	5.4	3	2.0	115	143	1	1	0	11
Rock Pond	5.9	11	0.8	57	301	0	0	—	–
Woods Lake (SL)	6.2	15	0.5	34	170	0	0	0	26

[a]0 = no liming record; 1 = limed more than 5 yr before coring date and/or water chemistry; 2 = limed less than 5 yr before coring and/or water chemistry (Bernert and Sullivan 1990).

Establishment of the Sediment Core Chronologies

^{210}Pb activity was measured in 13 or 14 specified intervals from the primary sediment core from each lake. The concentration of ^{210}Pb in the samples was measured by assay for the concentration of ^{210}Po using a slightly modified version of the method developed by Eakins and Morrison (1978), assuming secular equilibrium between the ^{210}Pb and ^{210}Po. The samples were counted between 8 and 36-h on low-level alpha spectroscopy systems. These measurements were carried out by Atomic Energy of Canada Limited at Chalk River, Ontario. The constant rate of supply (CRS) model, using the program described by Binford (1990), was used to generate ^{210}Pb dates. The CRS model assumes that the flux of ^{210}Pb to the sediment surface is constant through time (Appleby and Oldfield 1983). If the bulk rate of sediment accumulation changes, then sedimentary ^{210}Pb will be diluted or concentrated (Appleby and Oldfield 1983). Error estimates for individual ^{210}Pb dates were calculated based on the Poisson distribution, as described by Binford (1990).

Of the original 23 lakes in this study, two were eliminated from further analyses because their ^{210}Pb profiles indicated significant mixing in the uppermost sediment (Woods Lake (SL) and St. John Lake), and one additional lake (Rock Pond) was eliminated because of preservation problems with the chrysophyte scales. Of the 20 remaining cores, ^{210}Pb dates showed that the standard deviations (SD) for inferred dates after 1970 were <1 yr, with the exception of those calculated for Fourth Lake Bisby where the SD was 3.3 yr. The median SD for the bottom intervals was approximately 8 yr. Detailed information on the ^{210}Pb profiles and individual error estimates can be found in Cumming (1991).

Laboratory Methods

A known mass of wet sediment was digested in a mixture of sulfuric acid and potassium dichromate from selected sediment core intervals (usually 0–0.25, 0.5–0.75, 1–1.25, 1.5–1.75, 2–2.25, 3–3.25, 4–4.25 and 5–5.5, 7.5–8, 10–11, 12–13, 15–16, 17–18 and 20–21 cm). Following digestion of the organic matrix, the samples were centrifuged and repeatedly washed with distilled water until the material was acid free (see Smol 1983 for details). A known volume from each suspension was then diluted with distilled water and poured into evaporation trays (Battarbee 1973), where the microfossils were allowed to settle onto microscope coverslips. Once dry, the coverslips were mounted onto glass slides using Hyrax®. Four slides were produced for each sample processed.

Chrysophyte scales were counted along parallel transects utilizing oil immersion (1250× magnification, NA = 1.3) and phase contrast optics. In general, at least 500 scales were enumerated from each sample. Enumeration of chrysophyte scales using light microscopy is a practical and reli-

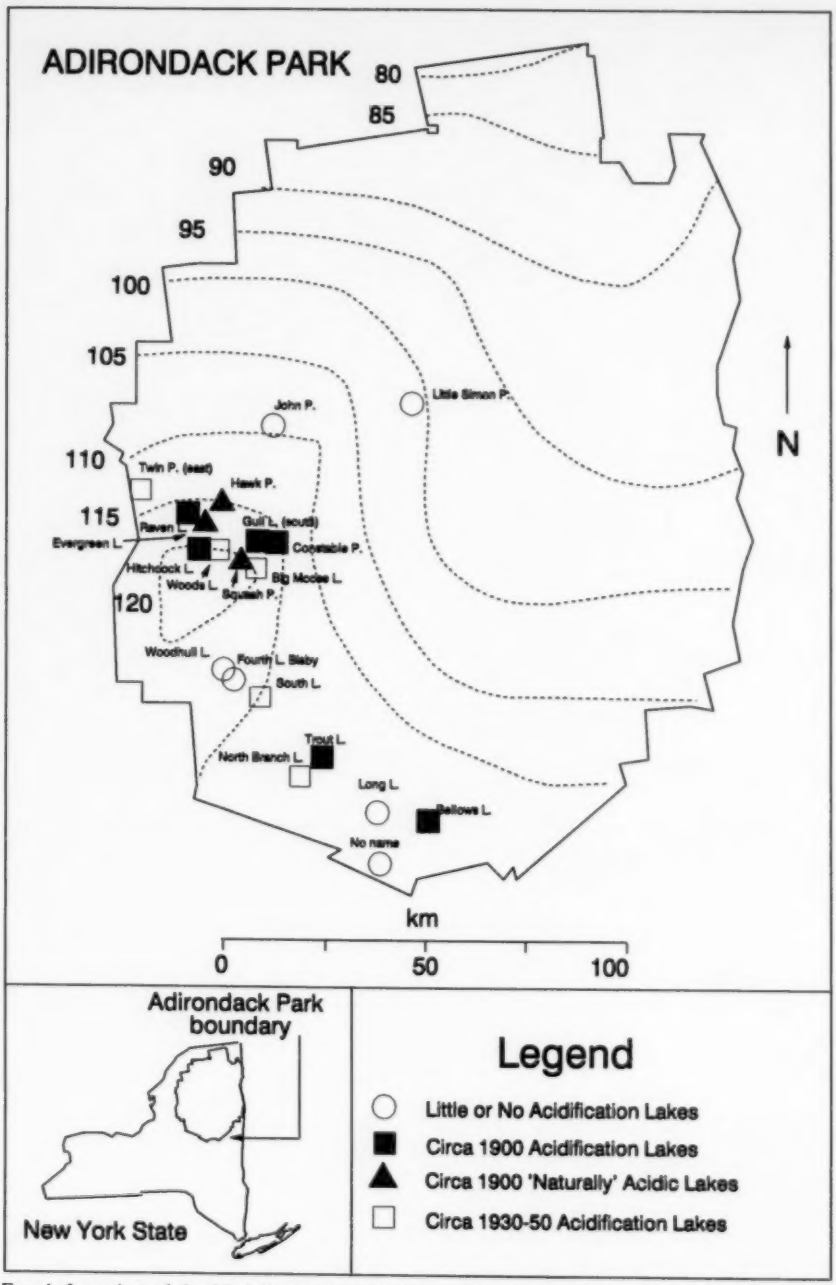

FIG. 1. Location of the 20 Adirondack study lakes. The lakes are coded into four groups that were determined based on a cluster analysis of the inferred-pH profiles since preindustrial times. Precipitation contours are shown in 5 $cm \cdot yr^{-1}$ intervals (Sullivan et al. 1990). Because sulfate concentrations in rainfall are relatively uniform across the Adirondacks, the amount of precipitation is a good proxy for sulfate loadings (Driscoll et al. 1991).

able way to identify and count large numbers of scales, provided that high-resolution optics and an appropriate mounting medium are used (Smol 1986). The Adirondack chrysophyte flora has been well studied (e.g., Smol et al. 1984a; Siver 1991; Cumming et al. 1992a). Chrysophyte taxonomy follows references listed in Cumming et al. (1992a, 1992b).

Quantitative pH-Inference Methods and Error Estimation Techniques

The transfer function we used to infer lake water pH is based on a slight modification of the transfer function derived from the relationship between the measured lake water chem-

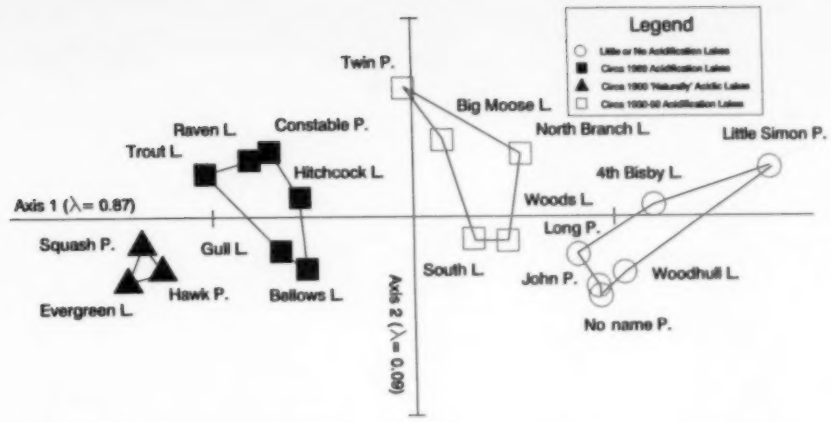

FIG. 2. PCA of the inferred-pH profiles from the 20 lakes. The lakes are coded according to their inferred-pH profiles and are grouped into four groups based on the results of the cluster analyses.

istry and the distribution of scaled chrysophyte taxa found in the surface sediments of 71 deepwater sediment cores (Cumming et al. 1992a). The transfer functions are based on weighted-averaging regression and calibration with tolerance downweighting (Line et al. 1994), but the new transfer function uses ln (y + 1) species transformation rather than the raw relative abundance data. This modification increased the predictive ability of the transfer function (r^2 = 0.77) and reduced the standard error of prediction to approximately 0.5 of a pH unit, an improvement of approximately 20% over the previous model (Cumming and Smol 1993).

Individual error estimates for each sample in this paper were provided by a bootstrap root mean squared error of prediction (bootstrap RMSE). These estimates were derived using the computer program WACALIB 3.3 (Line et al. 1994). The bootstrap RMSE provides an error estimate for each sample that consists of (1) a constant that represents the error due to the natural variation of different modern chrysophyte assemblages from which the predictive model was developed and (2) a unique error that captures the variability in the estimated taxon parameters for each sample (Birks et al. 1990a, 1990b).

Ordination and Classification of the Inferred-pH Profiles

To objectively recognize and classify the inferred-pH profiles, principal components analysis (PCA) and cluster analyses were run on the 20 pH profiles based on 17 pH values per core. These values were obtained from interpolations and extrapolations of the pH profiles where necessary, at 10-yr intervals between 1850 and 1970 and at roughly 5-yr intervals since 1970 (1975, 1980, 1985, and 1988). The PCA of the 20 pH profiles was based on a covariance matrix between variables (i.e., the inferred-pH values for the 17 times). Several hierarchical cluster analysis techniques, based on a squared Euclidean distance, were used: nearest neighbor, furthest neighbor, weighted group average, unweighted group average, median, centroid, and minimum variance. The cluster analyses were performed with the computer program CLUSTER (version 2.60, H.J.B. Birks and J.M. Line, unpublished program).

We used canonical variates analysis (CVA) to determine if any of the measured physical or chemical characteristics (those variables listed in Tables 1 and 2, with the exception of the nominal variables, as well as the inferred pH from 1850 and 1988), could discriminate between the four groups of pH profiles that were identified by the cluster analyses. Woods Lake (BM) and Little Simon Pond were eliminated from the CVA analyses because these lakes had been included in large-scale liming projects (Porcella 1988, 1989; McAvoy and Driscoll 1989). With the exception of elevation, pH, total Al, and dissolved organic carbon (DOC), all of the environmental variables were log transformed prior to CVA in order to meet the assumption of normality. Following an initial CVA, the environmental variables with high variance inflation factors (VIFs) were eliminated because of problems associated with multicollinearity (ter Braak 1988). In order to identify a minimal number of explanatory environmental variables, only those environmental variables with significant t-values were accepted into the final CVA model. Environmental variables not included in the CVA model were then "passively" added onto the CVA ordination using transition formulae (ter Braak 1988), so that the relationships between all the environmental variables, the sites, and the cluster groupings could be easily examined. Monte Carlo permutation tests were used to test the significance of the first two CVA axes. PCA and CVA were performed using the computer program CANOCO, version 3.10 (ter Braak 1990a, 1990b).

Results and Discussion

When Did Adirondack Lakes Acidify?

The first axis of the PCA ordination effectively separated (λ = 0.87) the pH profiles from the 20 lakes (Fig. 2). Although these pH trajectories form a continuum, cluster analysis defined four groups of lake response to acidic deposition (Fig. 1 and 2). All of the cluster analysis techniques used consistently identified these four categories: (i) lakes that showed little or no evidence of lake acidification since preindustrial times or lakes that underwent limited acidification and have subsequently recovered (Fig. 3B and 4), (ii) lakes with preindustrial pH values between 5.5 and 6

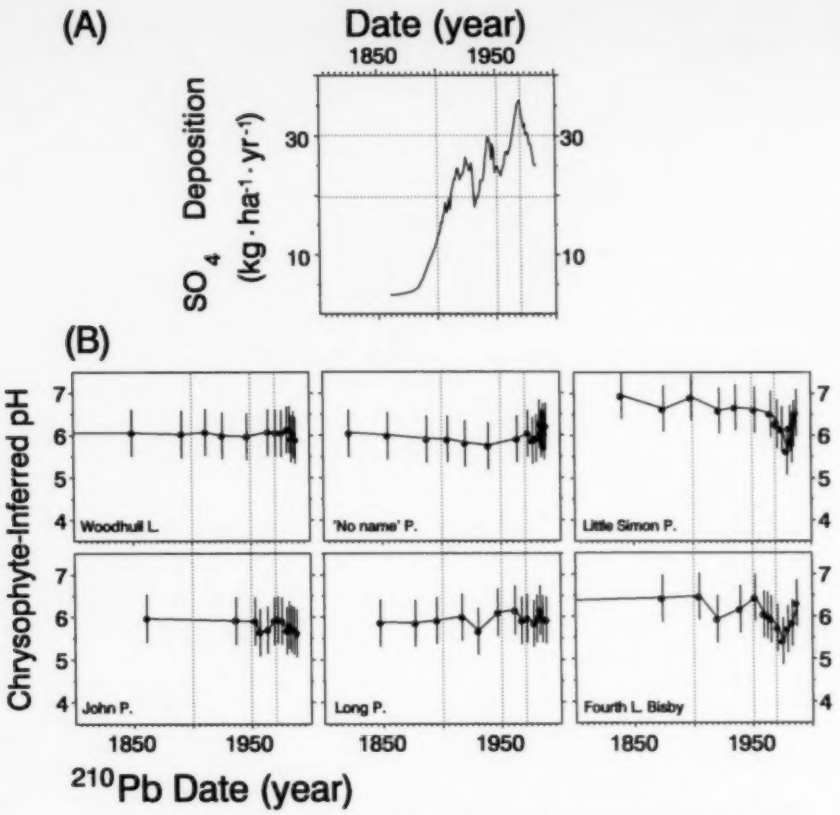

FIG. 3. (A) Historic wet sulfur deposition data for the Adirondack region (modified from Husar et al. 1991) and (B) inferred-pH profiles from the six lakes that were identified as a group of similar pH profiles by cluster analysis. These lakes showed little or no acidification since preindustrial times or have shown some evidence of recent acidification and subsequent recovery. Error bars represent the ±bootstrap RMSE.

that began to acidify ca. 1900 (Fig. 5A and 6), (iii) lakes that were "naturally" acidic but acidified further ca. 1900 (Fig. 5B and 7), and (iv) lakes with preindustrial pH values around 6.0 that acidified ca. 1930–50 (Fig. 8 and 9).

(i) Little or no acidification lakes

The four lakes that showed little or no evidence of acidification since preindustrial times (Woodhull Lake, John Pond, No name Pond, and Long Pond) are all located close together on the PCA ordination (Fig. 2). The inferred-pH profiles from these lakes show only small fluctuations since preindustrial times (Fig. 3). However, *Synura echinulata* Korsh. increased in all four lakes since 1900 (Fig. 4). This taxon has been noted as an "early-warning" indicator in lake acidification studies, as it is often one of the first species to increase with the onset of lake acidification (e.g., Dixit et al. 1990). Post-1900, post-1950, and post-1970 increases in the relative abundance of *Synura petersenii* Korsh. also occur in Woodhull Lake, Long Pond, and No name Pond (Mud Pond), respectively (Fig. 3). In addition, post-1970 increases in *Mallomonas caudata* Ivanov *em.* Krieger occur in Long Pond and No name Pond (Fig. 3). *Synura petersenii* and *M. caudata* have a wide tolerance to lake water pH, but are extremely sensitive to concentrations of inorganic monomeric Al above 2 μM (Cumming et al. 1992a). These floristic changes are between species with similar pH optima, and therefore resulted in little or no change in inferred pH (Fig. 3). However, it is possible that other limnological variables, besides pH, have changed in these lakes. These "little or no acidification" lakes had preindustrial inferred-pH values around 6 and are located at relatively low elevations (485–572 m, median = 555.5 m). With the exception of Woodhull Lake, these lakes are located outside the region of highest sulfate deposition, as indicated by the precipitation isopleths (Fig. 1). Woodhull Lake is an extremely large and deep lake in comparison with others in this dataset (Table 1) and therefore has a larger intrinsic ability to neutralize acidic inputs when compared with lakes with smaller volumes.

Little Simon Pond and Fourth Lake Bisby are grouped with the above lakes, but they are ordinated further to the right in the PCA (Fig. 2). Both of these lakes have undergone pH declines since preindustrial times, but the pH never declined below 5.5. Little Simon Pond shows large increases in the relative abundance of *S. echinulata*, and both Little Simon Pond and Fourth Lake Bisby show increases, albeit small, in the acidophilic taxon *Mallomonas hamata* Asmund whilst *M. caudata* declined. The inferred background pH values in these lakes were >6 (Fig. 3) and the inferred-pH values

never fell below 5.5. Little Simon Pond and Fourth Lake Bisby are both located outside the region of highest sulfate deposition (Fig. 1). Interestingly, the pH trajectory for Fourth Lake Bisby (Fig. 3B) was inversely related to the sulfate deposition (Fig. 3A), suggesting that this lake may be extremely sensitive to changes in sulfate deposition.

(ii) ca. 1900 acidification lakes

Lakes in this category started to acidify ca. 1900 (Fig. 5), concurrent with the dramatic increase in sulfate deposition (Fig. 3A). Preservation of chrysophyte scales was excellent in all these lakes with the exception of pre-1900 sediment from Gull Lake South. These lakes had preindustrial pH values between 5 and 6 and underwent inferred-pH declines of ~0.75–2 pH units. The rates of the pH decline were highly variable (Fig. 6). In general, the post-1900 floristic shifts in chrysophyte species composition were from the native *Mallomonas duerrschmidtiae* Siver, Hamer and Kling to more acidophilic taxa such as *M. hamata* and *Mallomonas hindonii* Nicholls (Fig. 6). *Mallomonas hindonii* is widely recognized as an excellent indicator of very acidic waters with high monomeric Al concentrations (Cumming et al. 1992a). *Mallomonas hamata* is also a good indicator of acidic conditions, but this taxon has a larger tolerance to pH when compared with *M. hindonii* (Cumming et al. 1992a). Many lakes in this group also show post-1900 increases in *S. echinulata* and *Synura sphagnicola* Korsh., usually preceding or simultaneous with increases in *M. hamata* and *M. hindonii*.

These lakes are relatively small (10–47 ha, median = 16 ha), shallow (maximum depth 4–24 m, median = 7.5 m), and from high elevations (546–651 m, median = 585 m) (Table 1). With the exception of Trout Lake and Bellows Lake, these lakes receive the highest present-day sulfate loadings in the Adirondacks (Fig. 1). Trout Lake has undergone an inferred-pH decline of >1.5 pH units whereas Bellows Lake has acidified less. Trout Lake is the highest elevation lake in this study and receives a greater sulfate load than is indicated by the coarsely defined precipitation isopleths in Fig. 1.

(iii) ca. 1900 "naturally" acidic lakes

This category consists of three lakes that were naturally acidic (inferred pH < 5.5), but acidified even more ca. 1900 (Fig. 5B and 7). In comparison with the ca. 1900 acidification lakes, these three are generally smaller (2–18 ha, median = 13 ha), more shallow (maximum depth 4–18 m, median = 7 m), and from higher elevations (613–650 m, median = 645 m). All three lakes are located in the region that receives the highest sulfate deposition load in the Adirondacks (Fig. 1). The floristic shifts that occurred in these lakes since preindustrial times were similar to those observed in the ca. 1900 acidification lakes. One exception was in Squash Pond, where post-1970 increases were also observed in the acidophilic chrysophyte *Chrysodidymus synuroideus* Prowse (Cumming et al. 1992a).

(iv) ca. 1930–50 acidification lakes

The five lakes in this category started to acidify ca. 1930–50 (Fig. 8). The inferred background pH values in these lakes range from ~5.7 to 6.3 (Fig. 8). As was observed in the lakes that started to acidify ca. 1900, the magnitude and rates of pH change in these lakes were variable. Although the largest changes in inferred pH occurred ca. 1930–50, floristic changes began in these lakes ca. 1900, as indicated by increases in *S. echinulata*, *S. sphagnicola*, *S. petersenii*, and *Mallomonas* "small" (Fig. 9). Although such changes resulted in little overall change in inferred pH, they are indicative of the first stage of acidification. The large declines in pH ca. 1930–50 were the result of floristic changes to higher relative abundances of *M. hamata* and *M. hindonii* whilst *M. duerrschmidtiae* (Fig. 9) declined. The inferred-pH profiles of North Branch Lake, Twin Pond, and Big Moose Lake were more similar than those of South Lake and Woods Lake, as illustrated in the PCA ordination (Fig. 2). The five lakes in this category are generally larger (5–520 ha, median = 23 ha), deeper (maximum depth range 8–20 m, median = 13 m), and lower in elevation (413–615 m, median = 575 m), with larger watershed areas (33–9555 ha, median = 184 ha) and higher preindustrial pH values, when compared with the lakes that acidified ca. 1900.

Can Acidic Deposition Account for the Observed Acidification Patterns?

All of the study lakes show evidence of post-1850 changes in chrysophyte species composition; 80% of these changes were clearly indicative of large declines in lake water pH since preindustrial times. Three major hypotheses have been proposed to account for the large number of acidic lakes in the Adirondacks and elsewhere: (1) natural processes (e.g., Pennington 1984), (2) watershed disturbances and associated vegetational and soil changes (e.g., Rosenqvist 1978, 1980; Krug and Frink 1983; Krug 1989; Dobson et al. 1990), and (3) atmospheric deposition of strong acids (e.g., Turner et al. 1990). These hypotheses can be assessed by comparing the logical consequences of each hypothesis in light of the inferred-pH profiles (e.g., Charles et al. 1989).

Studies on postglacial development of North American and European lakes show that acidic to weakly alkaline lakes have undergone a gradual long-term acidification. Rates of pH changes in the Holocene are estimated to have been slow, usually less than 0.5 pH units per 1000 yr (Renberg and Hellberg 1982; Christie and Smol 1986; Whitehead et al. 1986, 1989; Atkinson and Haworth 1990; Birks et al. 1990b; Renberg 1990). Long-term acidification studies are reviewed elsewhere (e.g., Battarbee 1984; Charles and Norton 1986; Charles et al. 1989; Battarbee et al. 1990). Because estimated rates of inferred-pH declines reported here are much faster than those that have occurred during natural ecosystem development, natural long-term processes cannot explain the recent acidification in the Adirondacks.

Logging, fire, forest blowdown, and similar disturbances can cause significant pH changes in a lake, but such changes are usually relatively short-lived (e.g., Nilsson et al. 1982; Rhodes 1991). The response of a lake to a watershed disturbance is related to the nature, extent, and severity of the disturbance, the past frequency of disturbances, the physical characteristics of the watershed (geology, soils, vegetation, slope, etc.), and limnological variables, as well as the taxonomic composition and rate of vegetational regrowth (Charles et al. 1989). Documented responses following logging vary from a short-term decrease in pH (Bormann and Likens 1979) to an initial rapid increase in pH, followed by a gradual pH decline to predisturbance levels (e.g., Nilsson et al. 1982). Rhodes (1991) observed the latter pattern of disturbance during an investigation of the effects of forest fires on an acidic Maine lake.

Recently, Dobson et al. (1990) proposed that forest blowdown may have contributed to the recent lake acidification

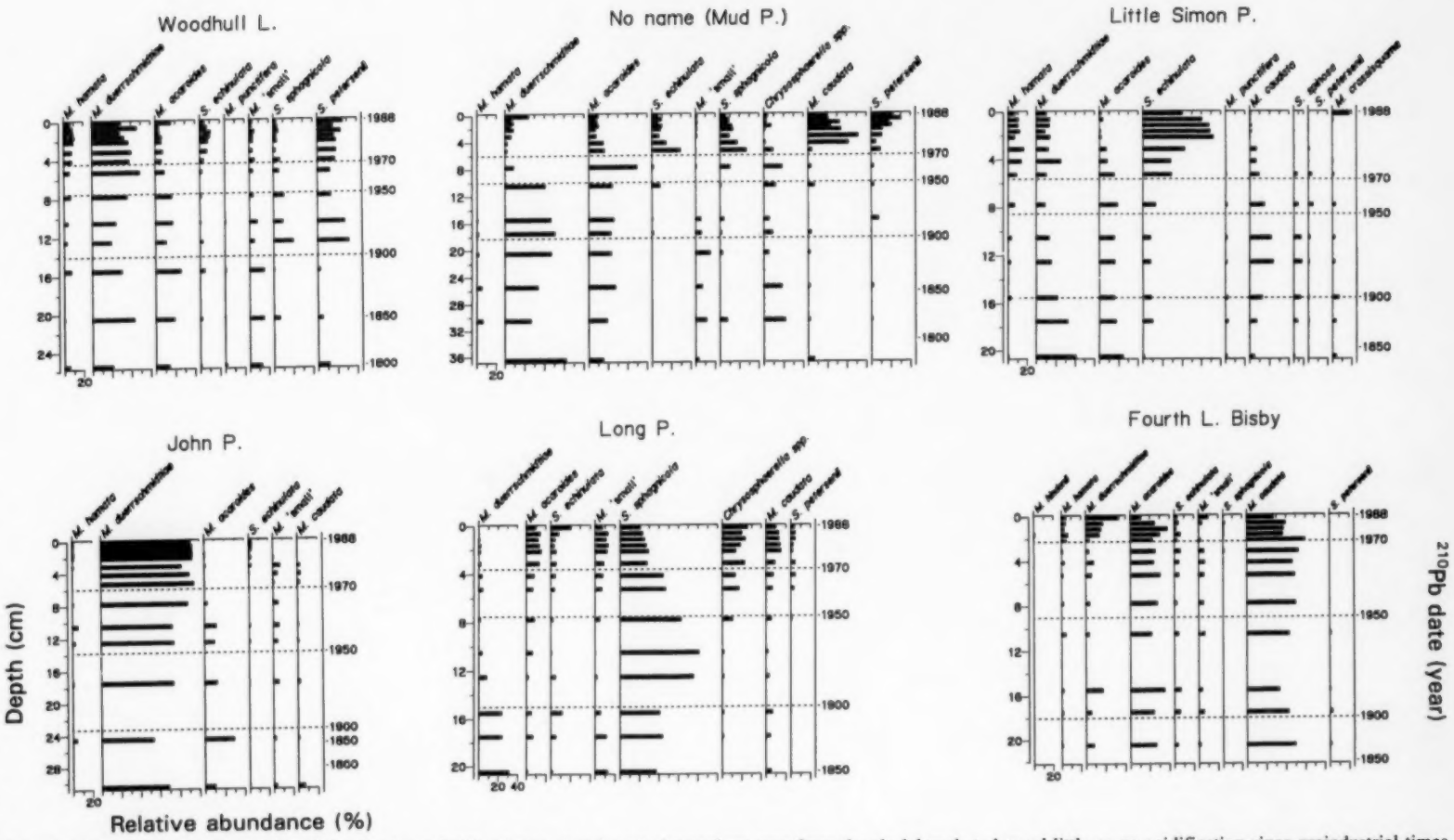

FIG. 4. Sediment core stratigraphies of the dominant (>1% relative abundance) chrysophyte taxa from the six lakes that showed little or no acidification since preindustrial times or have shown some evidence of recent acidification and subsequent recovery (see Fig. 3 for inferred-pH profiles).

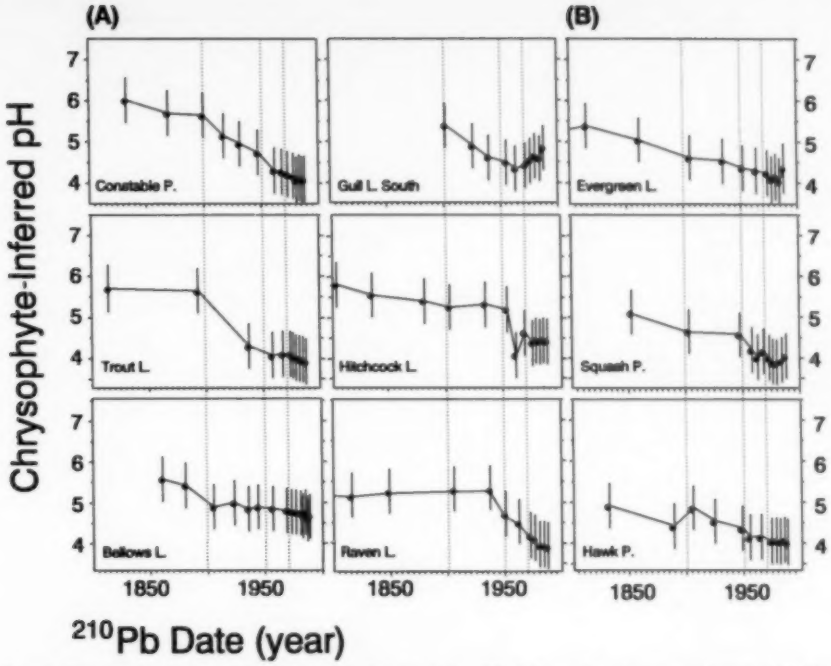

FIG. 5. Groups of similar inferred-pH profiles as identified from the cluster analyses. (A) Six lakes that had preindustrial pH values between 5 and 6 and underwent substantial decline in pH since ca. 1900; (B) three lakes that had preindustrial pH values <5.5 and have acidified further since 1850. Error bars represent the ±bootstrap RMSE.

in the Adirondacks by the formation of hydrologic pathways that would limit the contact of water with the soils and bedrock, thereby limiting the potential neutralization of the surface runoff. The main support for this theory as a causal factor that may cause or enhance lake acidification was a significant correlation between the estimated extent of the 1950 forest blowdown in individual watersheds and the associated present-day measured lake water pH in Adirondack lakes, as well as a temporal association between declining pH trends and forest blowdowns in three Adirondack lakes (Dobson et al. 1990). Dobson et al. (1990) used the sum of the estimated percentage of light, medium, and heavy blowdown as an overall index of blowdown (LMH). The results presented in this study and those of Charles et al. (1990) provide additional information on the temporal trends in pH for a number of additional Adirondack lakes that were also assessed by Dobson et al. (1990). We conclude that there are a number of inconsistencies with forest blowdown being an important causal mechanism of recent lake acidification. First, some low-pH lakes that had significant forest blowdown have not acidified (e.g., John Pond, LMH = 100). Second, in many lakes, the onset of acidification began prior to the forest blowdown. For example, Squash Pond (LMH = 10), Woods Lake (BM) (LMH = 100), Constable Pond (LMH = 5), and Hawk Pond (LMH = 30) all started to acidify well before the reported blowdowns, and the rates of acidification in these lakes are not related to the severity of the reported blowdowns (i.e., the larger the blowdown, the greater the expected acidification). In addition, Charles et al. (1990) report that Upper Wallface Pond (LMH = 80), Merriam Lake (LMH = 100), and Barnes Lake (LMH = 0) all started to acidify ca. 1900. Of the Adirondack lakes with estimates of both forest blowdown and temporal pH trends, less than one third are consistent with the blowdown acidification theory. Additional evidence supporting the assumption that watershed disturbances are not a major mechanism related to recent lake acidification is found in Charles and Norton (1986), Battarbee et al. (1990), Sullivan (1990), and Charles et al. (1991). Based on the lack of consistency between the timing of the blowdowns and the expected intensity of the response, we conclude that the blowdown theory cannot account for the recent widespread acidification of Adirondack lakes, although it may play a minor role in some lakes.

Atmospheric deposition of strong acids has increased dramatically in the Adirondacks and many areas of North America since preindustrial times (Husar et al. 1991). Wet sulfate deposition in the Adirondacks has increased since ca. 1870, except for the intervals during the Great Depression and following World War II (Husar et al. 1991; Fig. 3A). Sulfate deposition reached a high of ~35 $kg \cdot ha^{-1} \cdot yr^{-1}$ by 1970, after which there was a decline of ~30%, corresponding to the reduction in the S emissions in the northeastern United States and Canada (Fig. 3A). It has been estimated that the concentration of sulfate in many Adirondack lakes may be five times higher than before 1800 (Galloway et al. 1983; Holdren et al. 1984).

The response of a lake to acidic deposition depends on its ability to neutralize the acidic inputs through watershed and/or in-lake processes. Each lake/watershed system is highly individualistic in terms of its geology, soils, hydrology, vegetation, and other factors. It is therefore not sur-

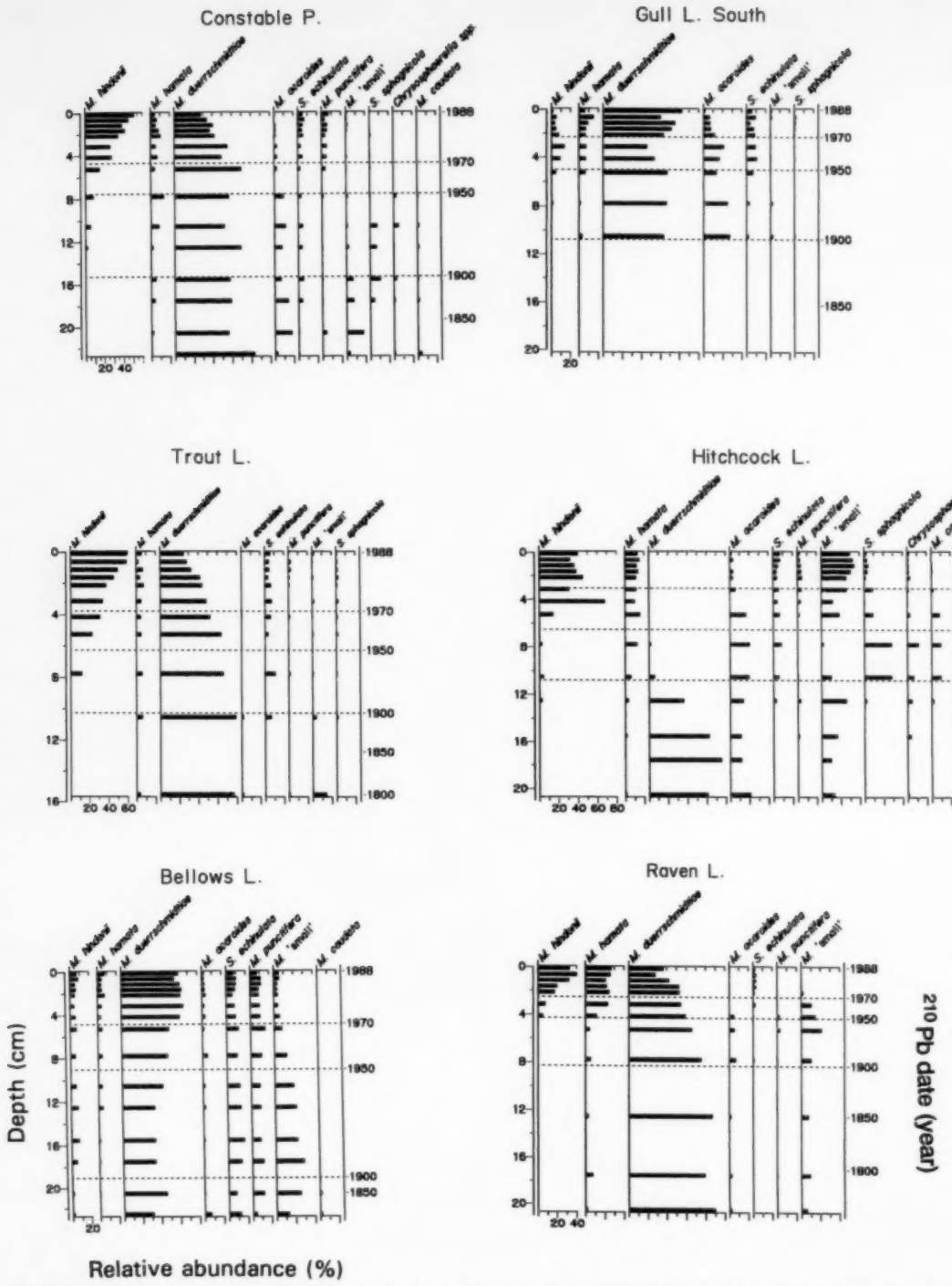

FIG. 6. Sediment core stratigraphies of the dominant chrysophyte taxa from the six lakes that had preindustrial pH values between 5 and 6 and underwent substantial declines in pH since ca. 1900 (see Fig. 5A for inferred-pH profiles).

prising that we observed a wide range of pH profiles in our lakes. CVA identified preindustrial inferred pH, present-day inferred pH, and elevation as the three variables that could best discriminate between the four groups of pH profiles (Fig. 10A). The eigenvalues of axes 1 (0.87) and 2 (0.49) and the species–environmental correlations for axes 1 (0.93)

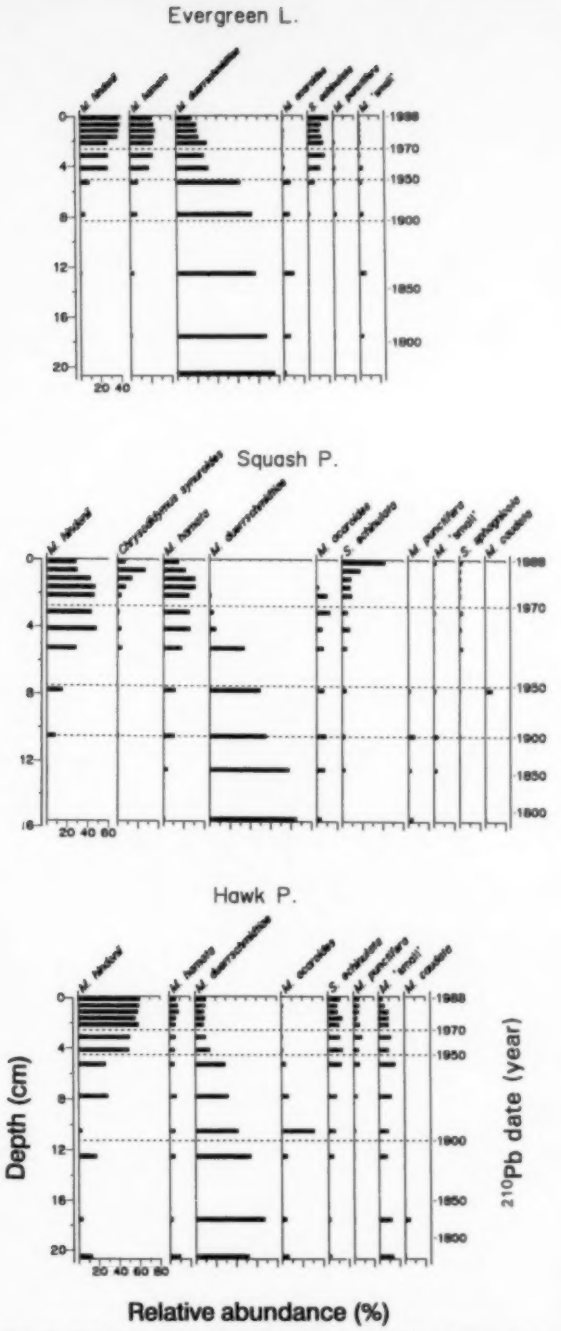

FIG. 7. Sediment core stratigraphies of the dominant chrysophyte taxa from the three "naturally" acidic lakes that acidified further since ca. 1900 (see Fig. 5B for inferred-pH profiles).

and 2 (0.70) are fairly high and captured 45% of the variance in the cluster groupings. However, Monte Carlo permutation tests (99 unrestricted permutations of axis 1, 99 unrestricted permutations of axis 2 with axis 1 as a covariable) show that axis 1 was significant ($p = 0.01$) whereas axis 2 was not ($p = 0.15$). The length and position of the biplot

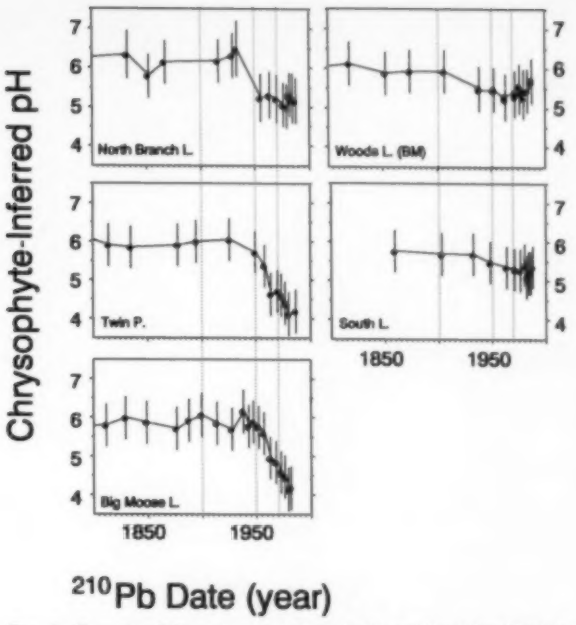

FIG. 8. Groups of five similar inferred-pH profiles identified by cluster analysis. These lakes started to acidify ca. 1930–50. Error bars represent the ±bootstrap RMSE.

arrows representing the inferred present-day and 1850 pH values, relative to the other correlated variables, show that these are the most important contributors to axis 1 (Fig. 10B). The lakes that "acidified ca. 1900" are effectively separated from the "little or no acidification" lakes based on either the 1850 or present-day inferred-pH values (Fig. 10A). These higher pH lakes are also associated with lower precipitation and, consequently, lower sulfate deposition loads (Fig. 10B). The ca. 1930–50 and ca. 1900 acidification lakes were similar in many respects (e.g., similar locations, sulfate loadings, and a great deal of overlap between many physical characteristics). However, the ca. 1950 acidification lakes had higher inferred present-day and preindustrial pH values and were generally larger, deeper lakes that had larger watershed areas when compared with the ca. 1900 acidification lakes (Fig. 10). The ca. 1900 "naturally" acidic lakes were among the highest elevation lakes and are positively associated with a high percentage of conifer vegetation and high concentrations of lake water DOC (Fig. 10B).

The observed pH profiles are consistent with the theory of lake acidification by acidic deposition. The observed patterns are plausible based on our understanding of lake/watershed acidification processes, and the observed changes are not consistent with alternative hypotheses of lake acidification. This study therefore lends support to and corroborates previous paleolimnological investigations and other studies that suggest that the acidification of Adirondack lakes has been caused mainly by acidic deposition from industrial sources (e.g., Charles et al. 1990; Sullivan 1990; Cumming et al. 1992b).

What Are Appropriate Critical Loads of Sulfate Deposition for Adirondack Lakes?

The term "critical load" is used to define the highest load

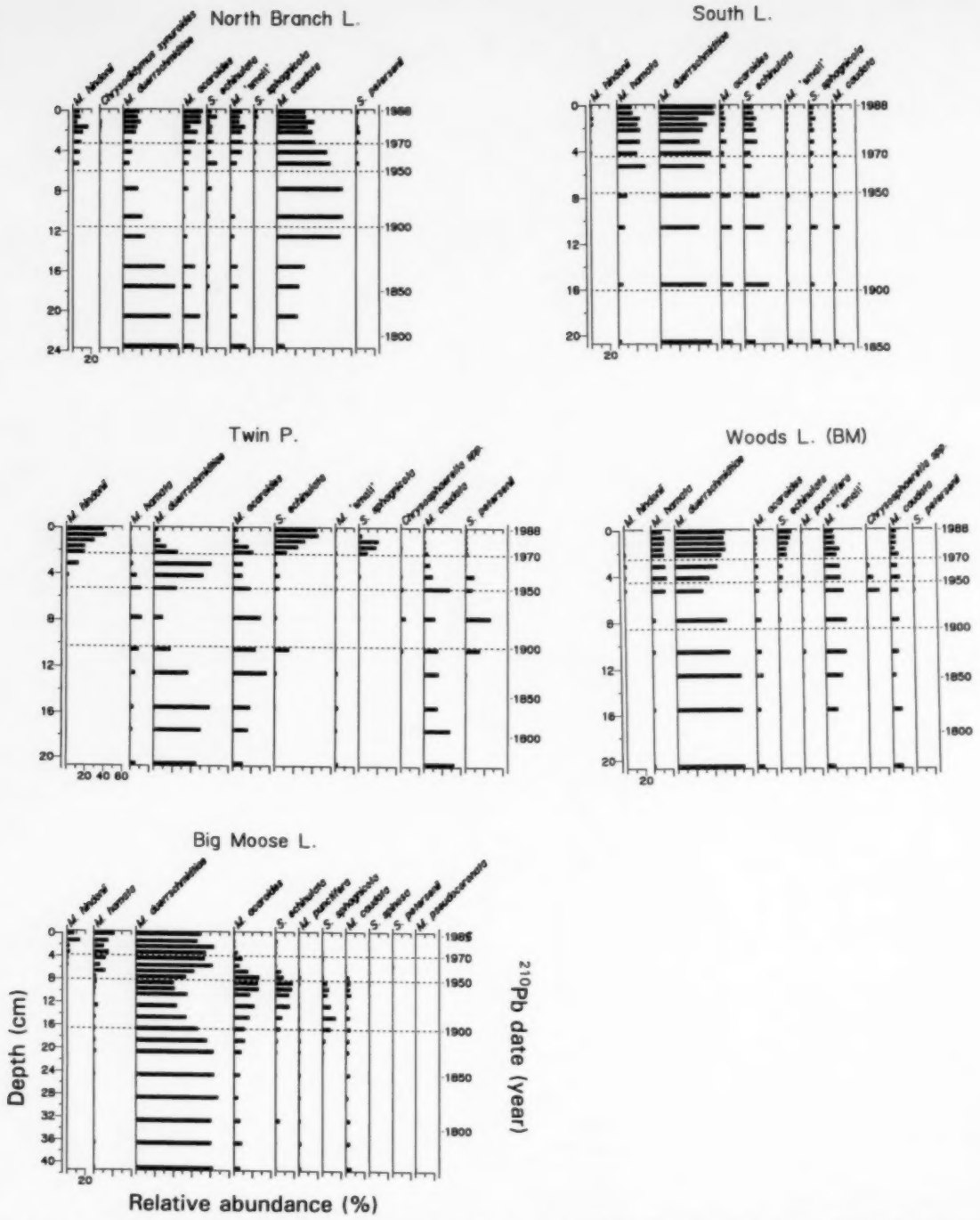

FIG. 9. Sediment core stratigraphies of the dominant chrysophyte taxa from the five lakes that acidified ca. 1930–50 (see Fig. 8 for chrysophyte-inferred pH from these cores).

of a substance that will not cause chemical changes leading to long-term harmful effects on even the most sensitive ecosystems (Nilsson 1986). It has been estimated that a wet sulfate load of 20 kg·ha^{-1}·yr^{-1} will only harm the most sensitive aquatic ecosystems (Canada/U.S. Memorandum of Intent, 1983). For lakes with critical load values in the range of current deposition in the northeast, model results differ by as much as 82 kg SO_4·ha^{-1}·yr^{-1} (Holdren et al. 1993). These differences are not only observed between different models, but also within models when slightly different approaches

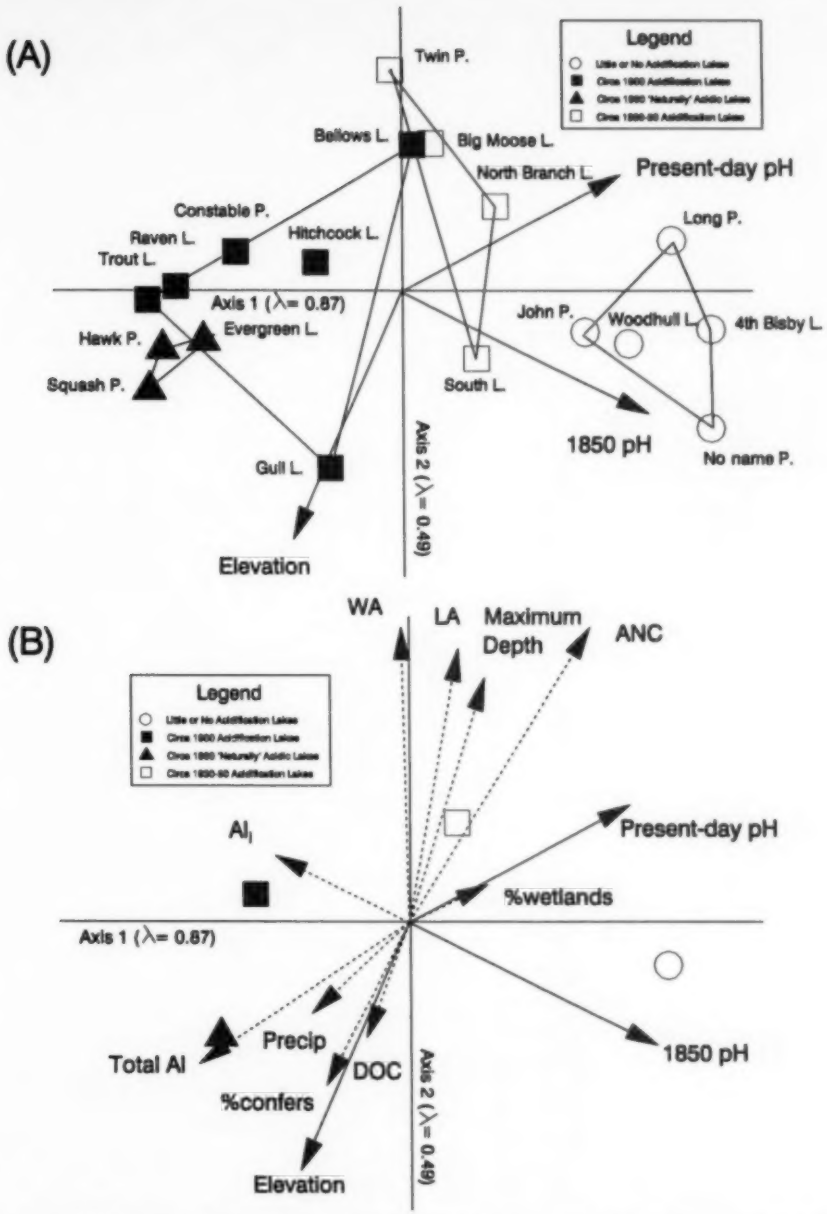

FIG. 10. CVA ordination plot showing (A) the lakes belonging to the various clusters in relation to the "active" environmental variables and (B) the "centroids" of the cluster groupings with respect to the "active" (solid arrows) and "passive" environmental variables (broken arrows) (see text for details; all abbreviations are defined in Tables 1 and 2).

are used for estimating certain parameters (Holdren et al. 1993). Due to the complex nature of lake acidification, estimates of critical loads derived from mechanistic approaches must be rigorously evaluated and substantiated with independent approaches. To date, estimates of critical loads have been derived from empirical relationships, budget studies, geochemical process-based models, and paleolimnological studies (Chadwick and Kuylenstierna 1991).

If we conclude that the recent widespread acidification of low-alkalinity Adirondack lakes is primarily due to acidic precipitation derived from the combustion of fossil fuels, our results can be used to estimate critical sulfate loads. By comparing when pH declines began in lakes and the corresponding estimates of sulfate deposition (Fig. 3A), critical loads of sulfate can be estimated. Based on such observations, we estimate that 5–10 and 20–25 $kg \cdot ha^{-1} \cdot yr^{-1}$ would be the critical loads for the lakes that started to acidify ca. 1900 and ca. 1950, respectively. We therefore should not expect

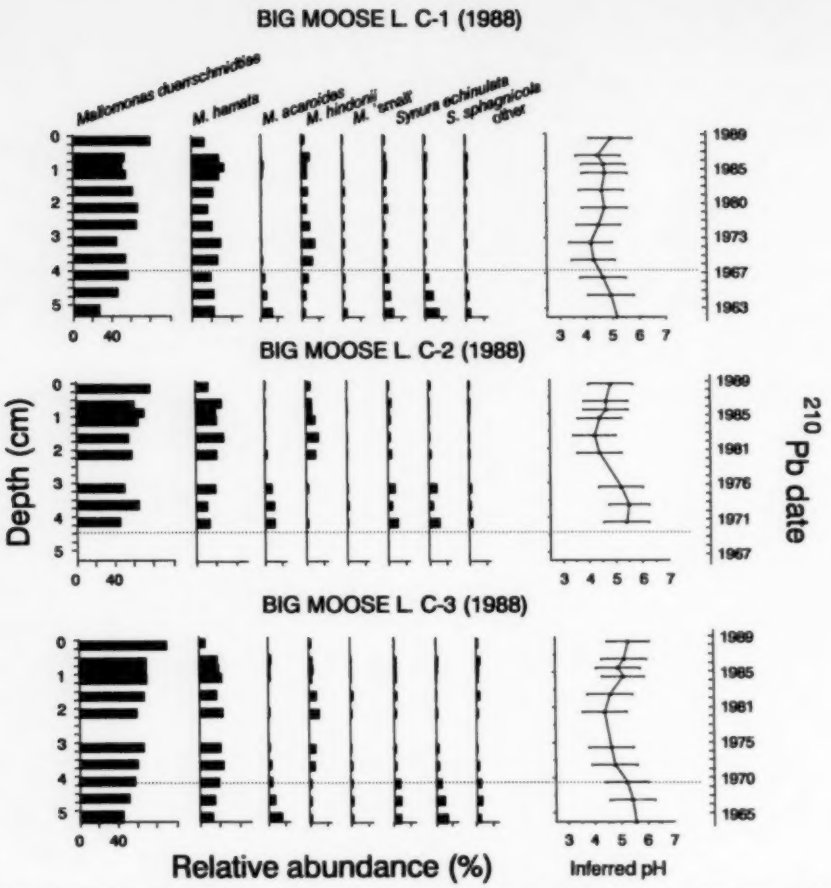

FIG. 11. Stratigraphies of the dominant chrysophyte taxa and the inferred-pH values in the top 5 cm of triplicate sediment cores from Big Moose Lake. Error bars represent the ±bootstrap RMSE.

reversals of acidification until sulfate deposition has been lowered to below these levels. These estimates assume that the relative proportions of strong acid anions and base cations have not changed over the past 100 yr, and that processes controlling the neutralization of precipitation today are similar to those in preindustrial times.

How Have Adirondack Lakes Responded to the Post-1970 Decreases in Sulfate Deposition?

Recent decreases in acidic sulfate loading have resulted in the reversal of lake acidification in whole-ecosystem experiments and in some lakes (see reviews by Wright and Hauhs 1991 and Schindler et al. 1991). For example, post-1970 increases in lake water pH following reductions in sulfate deposition have been documented in Canada (e.g., Dillon et al. 1986; Hutchinson and Havas 1986; Keller and Pitblado 1986; Dixit et al. 1989; Gunn and Keller 1990), Norway (Wright et al. 1988), Sweden (Forsberg et al. 1985), and Scotland (Battarbee et al. 1988; Allott et al. 1992), although it is unknown if these rates of chemical recovery can be extrapolated within similar regions, let alone to other regions. The effects of decreased sulfate deposition on Adirondack lakes are further complicated because large uncertainties exist in the dry deposition of acidic anions, base cations, and the wet deposition of nitrate (Driscoll et al. 1991), important factors that also regulate the acidity of surface waters.

With the exception of two lakes that have been the subject of massive mitigation (liming) projects and two lakes that have a disproportionately high amount of surrounding wetland, pH changes in Adirondack lakes since the post-1970 declines in sulfate deposition have been small and variable.

Post-1970 floristic changes in No Name Pond are indicative of a small pH increase (Fig. 3B and 4) whereas the other three lakes that have undergone "little or no acidification" since preindustrial times have been in a steady-state since 1970 (Fig. 3B). The other two lakes in this group, Little Simon Pond and Fourth Lake Bisby, have undergone post-1970 floristic changes that are indicative of pH recovery (Fig. 3B and 4). The post-1970 pH increases in No Name Pond and Fourth Lake Bisby may have resulted from the generation of alkalinity by sulfate reduction in the surrounding wetlands. No Name Pond and Fourth Lake Bisby are unique in that wetlands comprise a relatively large percentage of their watersheds (Table 2). The recent pH increase in Little Simon Pond is not surprising. Little Simon Pond was limed in August 1986 when 86 tons of calcite was

added which resulted in lake water calcite concentrations of 13 $mg \cdot L^{-1}$ (Porcella 1988, 1989). The chrysophyte assemblage clearly responded to this liming event, with decreases in the acid-tolerant *M. hamata* and increases in the higher pH and Al-sensitive taxon *M. crassisquama* Asmund (Fott) (Fig. 4). The inferred-pH values closely follow the yearly mean lake water pH measurements from this site (Cumming 1991). Fourth Lake Bisby was also limed, but this liming was minimal in comparison with the liming of Little Simon Pond (Table 2).

With the exception of the post-1970 pH increases in Gull Lake South, the post-1970 pH trends in the lakes that started to acidify ca. 1900 have been small (Fig. 5). However, Constable Pond, Trout Lake, Bellows Lake, Raven Lake, and Hawk Pond are still acidifying whereas Evergreen Lake, Squash Pond, and Hitchcock Lake show little consistent change (Fig. 5). The reason for the post-1970 inferred-pH increase in Gull Lake is not known.

The post-1970 pH trends in all the lakes that started to acidify ca. 1950 suggest that they have reached steady-state (Fig. 8 and 11). These observations are consistent with the estimates of critical loads for these lakes. However, the recent pH increases in Woods Lake are explained by the fact that it was limed in May 1985, which dramatically raised the lake water calcite concentration to 28 $mg \cdot L^{-1}$ (ANC = 450–550 $\mu eq \cdot L^{-1}$). Woods Lake, however, quickly reacidified to near 0 $\mu eq \cdot L^{-1}$ by September 1986 (Driscoll et al. 1989a, 1989b), at which time it was limed again. In the second liming, 35 tons of calcite was added, increasing lake water calcite concentrations to 44 $mg \cdot L^{-1}$. This second liming used several size ranges of powdered calcite, with the assumption that this liming would prolong the duration of the treatment. This liming event was picked up in the sedimentary record, but was not as strong as expected. Although the median measured pH increased and Al concentrations decreased, there were still large pH depressions and increases in Al associated with spring snowmelt (Driscoll et al. 1989a). Scaled chrysophytes tend to be most abundant in the spring, and therefore tend to reflect spring pH depressions. Moreover, because Woods Lake has a relatively slow sedimentation rate (i.e., the last 150 yr are represented by the top 12 cm of sediment), the uppermost 1 cm of sediment represents >10 yr of sediment accumulation. Consequently, the temporal resolution achievable from the sediment record from Woods Lake is low in comparison with our other lakes.

Triplicate cores taken from Big Moose Lake in 1988 all show post-1970 declines in *M. hamata* and *M. hindonii* and increases in *M. duerrschmidtiae*, suggesting that lake water pH has recently increased (Fig. 11). Although the inferred-pH increases are small (~0.3 of a pH unit), they are in agreement with monitoring data, suggesting that this lake may have started to recover from acidification (Sullivan et al. 1992).

Our observations of small but variable changes are consistent with the limited historical water chemistry measurements available from the Adirondack Long-Term Monitoring (ALTM) program (Driscoll and Van Dreason 1993). Based on data collected since the early 1980s, the 17 ALTM lakes have shown declines in lake water sulfate concentrations, although these lakes have not recovered with respect to lake water pH or ANC (Driscoll and Van Dreason 1993). The possibility that N leaching from the watershed might offset the benefits of sulfur dioxide reductions (Henriksen 1988; Henriksen et al. 1988) has been a matter of considerable concern. Based on ALTM data, Driscoll and Van Dreason (1993) suggested that such a scenario is probably occurring in the Adirondacks because nitrate concentrations have increased in most of the ALTM lakes, in spite of constant nitrate deposition.

Conclusions

Our paleolimnological results provide proxy data on the patterns of recent lake acidification in the Adirondacks. The lakes that started to acidify ca. 1900 and ca. 1950 were similar in many respects, but the former generally had lower preindustrial pH values, smaller surface and watershed areas, and shallower depths and were located at higher elevations. The lakes that showed little or no evidence of acidification all had preindustrial pH values of approximately 6 and were generally located outside the region of highest sulfate deposition. These pH trajectories are consistent with the responses postulated by the acidic deposition hypothesis and cannot be ascribed to natural causes or land-use changes. These data are in agreement with previous paleolimnological investigations and many other studies that suggest that recent lake acidification in the Adirondack Park has been caused mainly by acidic deposition from industrial sources.

Small and variable changes in pH have occurred in most of the study lakes since the post-1970 declines in sulfate emissions, in comparison with the overall acidification patterns. In general, lakes that began to acidify in the early 1900s continue to acidify or are in steady-state whereas the lakes that acidified ca. 1930–50 appear to be in steady-state or are showing small signs of pH recovery. These observations are in agreement with our estimates of critical sulfate loads. The lakes that showed large post-1970 increases in pH had either been the subject of massive mitigation projects (Woods Lake and Little Simon Pond) or have a disproportionately high amount of wetland (No Name Pond and Fourth Lake Bisby) in their drainage basins, possibly implicating sulfate reduction as a source of alkalinity generation in these lakes. Overall, these results suggest that Adirondack lakes have not been responsive to the post-1970 declines in sulfate emission, indicating that further emission reductions and more scientific investigations are necessary to understand changes that are now occurring in Adirondack lakes.

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Effects of Intermediate and Low Salinity Conditions on Growth Rate and Food Conversion of Atlantic Cod (*Gadus morhua*)

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Growth rates of Atlantic cod (*Gadus morhua*) were measured under different salinity conditions to test the hypothesis that growth would be best in an isosmotic environment. The results of two experiments (spring and autumn 1991) conducted at three different salinities (7, 14, and 28‰) and two feeding regimes indicate a significant effect of salinity and ration on growth rate. Within each experiment, growth rates were highest for cod maintained in intermediate salinity conditions (14‰). Growth rates in low salinity conditions (7‰) were higher than in seawater (28‰) during the spring, but during the autumn, growth rates of cod held under low salinity conditions and in seawater were similar. Higher growth rates at lower salinities resulted from an increase in food conversion efficiency. They were not associated with an increase in food intake, changes in composition (proteins, lipids, or water), or relative allocation of energy to the tissues (muscle, liver, and gonads) of cod. The results indicate that rearing cod at intermediate salinities, such as would occur in estuaries or coastal regions, could confer an advantage for cod aquaculture.

Le taux de croissance de la morue franche (*Gadus morhua*) a été mesuré sous différentes conditions de salinité pour vérifier l'hypothèse selon laquelle la croissance serait meilleure dans un milieu isosmotique. Les résultats de deux expériences (printemps et automne 1991) menées sous trois conditions de salinité (7, 14 et 28 ‰) et deux fréquences d'alimentation indiquent un effet significatif de la salinité et de la ration sur le taux de croissance. Dans chacune des expériences, les plus hauts taux de croissance ont été observés sous les conditions de salinité intermédiaire (14 ‰). Les taux de croissance mesurés à faible salinité (7 ‰) furent plus élevés qu'en eau de mer (28 ‰) lors de l'expérience du printemps alors qu'à l'expérience d'automne, les taux de croissance des morues à 7 et à 28 ‰ furent comparables. Les taux de croissance plus élevés observés aux plus faibles salinités sont dûs à des changements dans les taux de conversion. Ils ne sont pas associés à des changements dans la consommation, dans la composition (protéines, lipides ou eau) ni à des changements relatifs dans l'allocation d'énergie entre les tissus (muscle, foie et gonades) de la morue. Les résultats de cette étude indiquent que l'utilisation de conditions de salinité intermédiaire, comme celles pouvant se retrouver dans les estuaires ou les régions côtières, pourrait conférer un avantage pour l'élevage de la morue.

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The effects of salinity on the growth of anadromous species, especially salmonids (Canagaratnam 1959; Otto 1971; Shaw et al. 1975; MacLeod 1977; Brett 1979; Clarke et al. 1981; McKay and Gjerde 1985; McCormick et al. 1989; Morgan and Iwama 1991), have been examined and some studies have also been conducted on marine species known to occur in waters of low salinity during part of their life cycle (Peters and Boyd 1972; Hettler 1976; Gutt 1985; Malloy and Targett 1991). Almost no information, however, is available about the growth of marine teleosts at lower salinities. The results of studies of salinity tolerances and osmotic responses suggest that some marine species are euryhaline and may tolerate exposure to very low salinities (Woo and Wu 1982; Wu and Woo 1983).

Atlantic cod (*Gadus morhua*) tolerate exposure to low salinities, and some Northeast Atlantic cod stocks occur in brackish waters. For example, juvenile cod have been found to congregate in river estuaries in the United Kingdom (Riley and Parnell 1984), and cod occur in the brackish waters of the Baltic Sea (Westin and Nissling 1991). Northwest Atlantic cod stocks appear to be strictly marine, but they tolerate low salinities (Odense et al. 1966; Dutil et al. 1992; Provencher et al. 1993). No evidence was found that acclimation to salinity as low as 7‰ was stressful for cod (Dutil et al. 1992). Direct transfer to salinities below 7‰ may result in increased mortality (Provencher et al. 1993), but gradual decreases in salinity over several months resulted in mortality only at salinities below 3‰ (Odense et al. 1966).

Salinity has been hypothesized to change the energy available for growth via modification of the metabolic cost of iono- and osmoregulation. The usual hypothesis proposed is that energetic costs are lowest in an isosmotic environment and that the energy savings are sufficient to permit increased growth (Morgan and Iwama 1991). The metabolic responses of teleost fish to changes in environmental salinity do not, however, show any consistent patterns, and the optimal salinities for growth seem to differ among species, life stage, and season (Morgan and Iwama 1991).

During recent years, interest has increased for cod aquaculture based on seasonal farming of wild fish caught in coastal fisheries. The rearing of cod in coastal areas or estu-

TABLE 1. Mean length, weight, and condition factor (K) of Atlantic cod at the beginning of the spring and autumn experiments for the three salinities. Probability level of significance between salinities is represented by P.

	Salinity (‰)			
	7	14	28	P
Spring experiment				
Length (mm)	335 ± 4	335 ± 4	329 ± 4	>0.22
Weight (g)	301 ± 13	304 ± 15	276 ± 12	>0.54
K	0.77 ± 0.01	0.78 ± 0.01	0.74 ± 0.01	>0.33
n	76	75	74	
Autumn experiment				
Length (mm)	444 ± 5	443 ± 4	440 ± 4	>0.78
Weight (g)	720 ± 23	688 ± 21	688 ± 19	>0.67
K	0.81 ± 0.02	0.78 ± 0.01	0.80 ± 0.01	>0.88
n	88	88	83	

aries can subject them to changes of salinity, so information about the growth potential of cod under these conditions is desirable.

Two experiments were conducted to assess the growth potential of Atlantic cod at intermediate salinities. Cod were reared at three different salinities (7, 14, and 28‰) under two feeding regimes for periods of 9 wk, with the objectives of measuring food intake, growth rate, food conversion efficiency, and energy allocation at each salinity.

Materials and Methods

Cod were caught by beam trawl in the Gulf of St. Lawrence near Matane (Quebec, Canada) in June 1990 and 1991. A complete description of holding conditions is given in Dutil et al. (1992). Two experiments were conducted, the first from March to May 1991 (spring) and the second from October to December 1991 (autumn), each lasting 9 wk following a temperature acclimation period of 1–2 wk.

Experiments were conducted in 1.2-m^3 tanks, using three different salinity treatments (7, 14, and 28‰). Each salinity was obtained by mixing fresh and seawater using manually controlled flowmeters. Temperature was held at 10°C, with temperature, salinity, and dissolved oxygen recorded and adjusted daily. Dissolved oxygen levels ranged between 7.4 and 10.8 mg/L, with most recorded values being higher than 8 mg/L.

Two feeding regimes were tested at each salinity. Cod were fed to satiation either once a week or three times weekly. Food provided to each tank of cod was weighed, and after 1 h, uneaten food was removed and weighed. Cod were fed with coarsely chopped rainbow smelt (*Osmerus mordax*) in the spring experiment and capelin (*Mallotus villosus*) in the autumn experiment.

For each combination of feeding and salinity, replicate tanks were used. Each fish was anaesthetized, tagged, measured (fork length in millimetres), and weighed (grams) and then, 16–22 cod were transferred to each tank. Length and weight measurements were used to calculate condition factor of cod at the beginning of each experiment. Condition was expressed as Fulton's condition factor (K) with the formula

$$K = 100 \cdot W/L^3$$

where W is fish weight (grams) and L is fish length (centimetres) (Bagenal 1978).

No significant differences in length and weight were observed among salinities ($P > 0.22$; Table 1) or among tanks ($P > 0.06$) at the beginning of both experiments. Condition factors were also similar; no significant differences were observed among salinities within each experiment ($P > 0.10$; Table 1). Lengths and weights were compared by ANOVA on log-transformed data. Nonparametric Kruskal–Wallis tests were used to compare condition factors, as transformation of the data could not make the variances homogeneous.

Two fish were sacrificed from each tank at the beginning of the experiments to determine sex and maturity, to measure gonad, liver, and muscle weights (±0.1 g), and to collect blood, gonad, liver, and muscle samples. When the experiments ended, all fish were sacrificed and were treated as above. Blood samples, however, were collected from only four fish from each tank. Subsamples of fish were also taken at random from each treatment group for estimation of the energy contents of gonad, liver, and muscle tissue at the beginning and at the end of each experiment. Muscle weight was approximated as carcass weight (eviscerated) with head removed. Blood samples were centrifuged to separate plasma, divided into aliquots, and frozen for further analysis.

For each experiment, growth, condition, and liver and gonad indices were calculated for each combination of feeding and salinity. Growth was expressed as specific growth rate (SGR) using the formula

$$\mathrm{SGR} = 100 \cdot (\ln W_T - \ln W_t) \cdot (T - t)^{-1}$$

where $\ln W_T$ is the natural logarithm of the weight (grams) at time T, $\ln W_t$ is the natural logarithm of the weight at time t, and $(T - t)$ is the time (days) between weighings (Jobling 1988; Hopkins 1992). The hepatosomatic index (HSI) and gonadosomatic index (GSI) were calculated as

$$\mathrm{HSI} = (\mathrm{LW}/\mathrm{SW}) \cdot 100$$

$$\mathrm{GSI} = (\mathrm{GW}/\mathrm{SW}) \cdot 100$$

where LW, GW, and SW represent liver weight, gonad weight, and somatic weight (grams) (total weight less gonad weight), respectively.

Food intake and food conversion efficiency were calculated for each experimental tank. Food intake was calculated from the food consumed in each tank on a weekly basis and was expressed as the quantity of food eaten per day on a weight per weight basis (grams). Food conversion efficiency was cal-

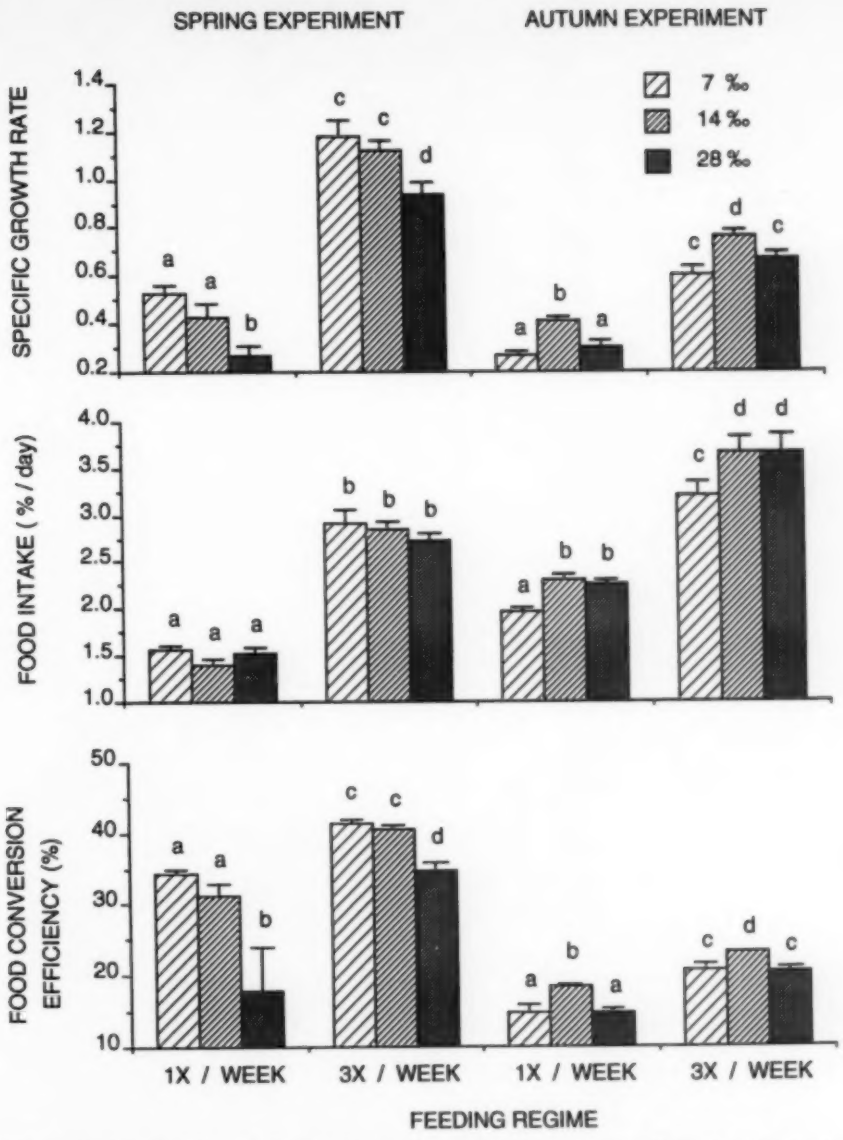

FIG. 1. Specific growth rates, food intake per day, and food conversion efficiencies of Atlantic cod at three different salinities (7, 14, and 28‰) and two feeding regimes (once and three times weekly). Mean values and standard errors for each salinity and feeding regime for both spring and autumn experiments are presented. Each value of specific growth rate represents 35–45 individuals, while corresponding values for food intake and food conversion efficiencies represent 18 and 2 observations, respectively. Within each experiment, values with the same letter are not significantly different (pairwise comparisons, $P > 0.05$).

culated for each tank by expressing the weight gain (grams) in each tank during the experiment as a proportion of total food consumption (grams) during the experiment. Mortalities during the experiments were taken into account in the calculations of food intake and food conversion efficiencies.

Plasma Na^+, Cl^-, and K^+ were measured using a multiple-ion analyser (Ciba Corning, model 644) and osmotic pressure determined with a microosmometer (Advanced Instrument, model 3MO). Analysis of Na^+, K^+, Cl^-, and osmotic pressure for the spring experiment did not reveal any significant differences (ANOVA, $P > 0.27$) between salinities. For that reason, only osmotic pressure was examined in the autumn experiment. Significant differences ($P < 0.001$) in osmotic pressure were observed between salinities for the autumn experiment. However, mean values were all in the range of 332–351 mosmol which is well in the range of values observed by Dutil et al. (1992) and for which there were no indications that acclimation was stressful for cod.

For each tissue, duplicate analyses of water, lipid, and protein content were performed on homogenized tissues.

TABLE 2. Condition factor (K), HSI, and GSI of Atlantic cod for each salinity at the end of each experiment for fish fed once (1×) and three (3×) weekly. Means, standard errors, and number of individuals (n) are presented. Probability level of significance between salinities is represented by P.

	Salinity (‰)			
	7	14	28	P
Spring experiment				
1×/wk				
K	0.84 ± 0.01	0.81 ± 0.01	0.78 ± 0.02	<0.017
HSI	3.30 ± 0.18	3.05 ± 0.36	2.88 ± 0.18	>0.497
GSI	0.25 ± 0.04	1.07 ± 0.54	0.62 ± 0.21	>0.095
n	32	30	30	
3×/wk				
K	0.95 ± 0.01	0.97 ± 0.02	0.92 ± 0.01	<0.049
HSI	5.62 ± 0.26	6.10 ± 0.22	5.22 ± 0.26	>0.051
GSI	0.84 ± 0.31	0.44 ± 0.08	0.69 ± 0.34	>0.577
n	32	29	30	
Autumn experiment				
1×/wk				
K	0.84 ± 0.01	0.87 ± 0.02	0.89 ± 0.02	>0.074
HSI	3.35 ± 0.16	3.65 ± 0.18	3.55 ± 0.20	>0.477
GSI	4.29 ± 0.49	3.44 ± 0.36	3.11 ± 0.34	>0.323
n	39	36	37	
3×/wk				
K	0.96 ± 0.02	0.96 ± 0.01	0.98 ± 0.01	>0.529
HSI	5.76 ± 0.17	6.03 ± 0.18	5.88 ± 0.20	>0.561
GSI	6.13 ± 0.52	6.63 ± 0.58	5.53 ± 0.60	>0.298
n	40	40	39	

Glycogen content was only analysed for the liver, since concentrations in the gonad and muscle of cod were assumed to be less than 0.4% of the wet weight (Kjesbu et al. 1991). Water content was determined by drying tissue samples to constant weight at 65°C. Lipid content was determined gravimetrically following Soxhlet extraction (Soxtec extraction unit 1043) with chloroform–methanol (2:1, v/v). Protein content was determined by evaluating nitrogen content using micro-Kjeldhal digestion followed by spectrophotometric determination using the Berthelot reaction (Haslemore and Rougham 1976). A correction was made for nonprotein nitrogen in muscle tissue using the method outlined by Niimi (1972). Glycogen was analysed by the enzymatic method described by Keppler and Decker (1983). Energy equivalents of 39.5, 23.6, and 17.1 kJ/g were assumed for lipid, protein, and glycogen, respectively (Kleiber 1975). Chemical compositions and energy contents of food (smelt and capelin) were estimated using the same methods.

Data are presented as means with standard errors. Comparisons of weight, condition, liver and gonad indices, growth rate, food intake, and conversion efficiency between treatments were performed using ANOVA. When significant differences ($P < 0.05$) were found, pairwise comparisons were made using Tukey–Kramer method (SAS Institute 1990).

Results

Growth Rate

Salinity and feeding regime had significant influences (two-way ANOVA, $P < 0.0001$) on growth rate in both spring and autumn experiments (Fig. 1). Specific growth rates at low and intermediate salinities (7 and 14‰) were similar to or higher than those obtained in seawater (28‰) and the responses to salinity were similar under both feeding regimes, although the pattern differed between seasons.

Specific growth rates at intermediate salinity (14‰) were always significantly higher ($P < 0.0001$) than in seawater (28‰). Growth rates at 14‰ were between 14 and 63% higher than in seawater depending on experiment and feeding regime. At low salinity (7‰), the growth responses differed between spring and autumn. Growth rates in the low salinity treatment (7‰) were significantly higher than in seawater (28‰) during spring, while no significant differences in growth rate between the two salinity conditions were observed during autumn (Fig. 1). In spring, growth rates of cod at low salinity and fed once and three times weekly were 98 and 26% higher than in seawater, respectively.

Growth rates observed during the autumn experiment were significantly lower ($P < 0.001$) than in the spring experiment. However, cod used in the autumn experiment were of a larger size ($P < 0.001$) than in the spring experiment (Table 1). Cod used in the spring experiment had a mean length of 333 mm and a mean weight of 293 g compared with a mean length and weight of 442 mm and 699 g, respectively, for cod in the autumn experiment. Condition factors were also significantly higher ($P < 0.002$) for cod used in the autumn experiment (Table 1). Both the spring and autumn cod had low HSI values (1.6–3.5%) and GSI values (0.3–1.8%) characteristic of immature cod or adult cod in a resting state.

Food Intake and Food Conversion Efficiency

Salinity had little effect on food consumption of cod, but food consumption was significantly influenced by feeding regime (Fig. 1). No significant differences (ANOVA, $P >$

TABLE 3. Mean (±SE) energy content (kJ/g) for muscle, liver, and gonads of Atlantic cod at the beginning (Initial) and at the end of each experiment for fish fed once (Final-1×) and three times (Final-3×) weekly. The number of individuals for each value varies between 3 and 9.

	Time and feeding regime	Salinity (‰)		
		7	14	28
Spring experiment				
Muscle	Initial	3.29 ± 0.12	3.22 ± 0.08	3.29 ± 0.06
	Final-1×	3.41 ± 0.06	3.45 ± 0.07	3.45 ± 0.04
	Final-3×	3.73 ± 0.07	3.75 ± 0.02	3.59 ± 0.03
Liver	Initial	17.61 ± 0.53	15.42 ± 0.79	14.54 ± 1.03
	Final-1×	20.93 ± 3.45	22.01 ± 2.66	18.96 ± 0.83
	Final-3×	26.20 ± 1.01	28.35 ± 0.62	26.29 ± 1.22
Gonads		—	—	—
Autumn experiment				
Muscle	Initial	3.24 ± 0.18	3.20 ± 0.10	3.24 ± 0.12
	Final-1×	3.44 ± 0.02	3.42 ± 0.04	3.51 ± 0.02
	Final-3×	3.89 ± 0.04	3.66 ± 0.07	3.67 ± 0.06
Liver	Initial	13.86 ± 3.37	11.60 ± 1.85	16.77 ± 4.10
	Final-1×	16.42 ± 0.83	17.22 ± 0.74	20.28 ± 1.88
	Final-3×	24.09 ± 0.96	25.35 ± 0.51	25.06 ± 0.98
Gonads	Initial	4.98 ± 1.19	3.58 ± 0.14	3.88 ± 0.10
	Final-1×	3.68 ± 0.10	4.60 ± 0.71	3.74 ± 0.66
	Final 3×	3.74 ± 0.10	4.09 ± 0.54	3.63 ± 0.06

0.18) in food intake per day on a weight per weight basis were observed among the three salinities in spring. However, food intake per day was significantly lower ($P < 0.05$) at 7‰ than at higher salinities in autumn (Fig. 1). For both experiments, food intake per day for cod fed once per week was significantly lower than for cod fed three times weekly (ANOVA, $P < 0.001$).

Food intake per day during the autumn experiment was significantly higher ($P < 0.001$) than during the spring experiment. However, smelt provided as food during the spring had a higher energy content (4.5 kJ/g) than capelin which was used as food during the autumn (3.5 kJ/g). Food intake per day expressed on an energy basis (kilojoules per kilogram per day) was very similar between the two experiments. Food intake per day for cod fed once and three times weekly during spring was 67 ± 11 and 128 ± 21 kJ/kg, respectively, while food intake per day for cod with the same feeding regimes during the autumn was 77 ± 9 and 124 ± 26 kJ/kg, respectively.

Significant differences (ANOVA, $P < 0.009$) in food conversion efficiency were observed between salinities, feeding regimes, and experiments (Fig. 1). In both spring and autumn, food conversion efficiencies at intermediate salinity (14‰) were significantly better than in seawater (28‰). At the lowest salinity (7‰), food conversion efficiencies during the spring were better than in seawater, but this was not so during the autumn. Food conversion efficiencies were also better for fish fed three times weekly than for fish fed once per week (Fig. 1). Finally, better food conversion efficiencies were observed during the spring experiment.

Final Condition

Salinity had no significant effect ($P > 0.08$) on final HSI and GSI (Table 2). Condition factor was lower at 28‰ ($P < 0.004$) for both feeding regimes in the spring but did not differ among salinities in the autumn.

Feeding regime had a significant effect ($P < 0.0001$) on final condition factor and HSI (Table 2); cod fed three times weekly reached higher condition factor and HSI values. In the spring experiment, GSI was comparable between the two feeding regimes, while in the autumn experiment, significantly higher ($P < 0.001$) GSI values were observed for cod fed three times weekly (Table 2). However, examination of GSI values for the two experiments indicates that cod in the spring experiment were in a resting state, while cod in the autumn experiment had begun gonad maturation.

Variations in Energy Content

No significant differences were observed among salinities in the proximate composition (protein, lipid, glycogen, and water contents) of muscle, liver, and gonads (ANOVA, $P > 0.1$). Estimations of muscle, liver, and gonad energy content for each salinity, as calculated from proximate composition, revealed no significant effect (ANOVA, $P > 0.17$) of salinity on energy composition (kilojoule per gram of tissue) in either experiment (Table 3). However, highly significant differences (ANOVA, $P > 0.0001$) were observed between the beginning and the end of the experiments and between the two feeding regimes for muscle and liver energy content (Table 3). Muscle energy content increased by 5–15% depending on the feeding regime between the beginning and the end of the experiments, while changes in the liver energy content for the same conditions ranged between 30 and more than 100%. Gonad energy content, which was estimated only during the autumn experiment, did not change ($P > 0.66$) over time during the experiment or between feeding regimes.

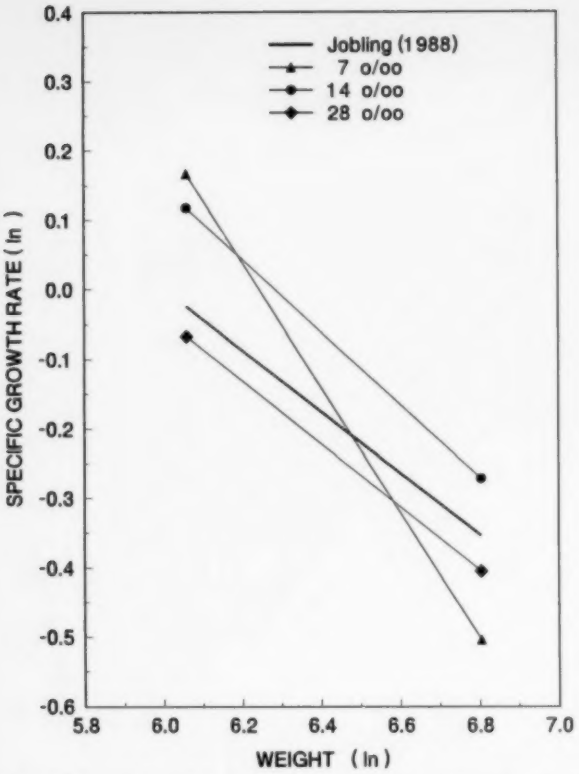

FIG. 2. Comparison of the relationship between specific growth rate and weight (natural logarithmic transformations) of Atlantic cod at a temperature of 10°C obtained from Jobling (1988) with those observed in this study at salinities of 7, 14, and 28‰. Lowest weights correspond to the results of the spring experiment, while largest weights represent the results of the autumn experiment.

Mortality

Few mortalities were observed in the experiments. In the spring experiment, 10 of 218 (5%) fish died, with six of these mortalities being the result of fish jumping out of the tanks. In the autumn experiment, 6 of 259 fish (2%) died.

Discussion

Effects of Salinity on Growth Rate

The results of this study indicate that salinity has a significant effect on the growth of cod and show that higher growth rates can be obtained in salinity conditions of 7 and 14‰ than in seawater (Fig. 1). Highest growth rates were also obtained for juvenile European flounder (*Platichthys flesus*) at intermediate salinities (5 and 15‰) (Gutt 1985). Juvenile flounder, however, are usually found in estuaries with water of low salinity. To our knowledge, no systematic studies have been done on the effects of salinity on the growth potential of marine teleosts, but it has been demonstrated that some marine fish species, including cod, can tolerate low salinities (Odense et al. 1966; Woo and Wu 1982; Wu and Woo 1983; Dutil et al. 1992; Provencher et al. 1993) or can survive longer periods of starvation when kept in lower salinity conditions (Woo and Murat 1981).

Significant differences in growth rate among salinities did not result from changes in food intake (Fig. 1) or from differences in the specific (per gram) energetic content among salinities. Furthermore, they did not result from changes in the relative allocation of energy to muscle, liver, and gonads (Tables 2 and 3). In other words, lower salinities enhanced production quantitatively and not qualitatively. However, studies on other fish species have demonstrated that changes in salinity could result in differences in the relative weight of liver (HSI) and in the protein, lipid, and water contents (percentage of fresh or dry weight) of liver and muscle (Arunachalam and Reddy 1979; Woo and Fung 1981). Feeding regime, on the other hand resulted not only in significant differences in growth rates, but also in differences in condition factor, HSI, and GSI. Similar relationships between ration, growth rate, condition, liver weight, gonad weight, and energy content have been observed previously (Edwards et al. 1972; Jones and Hislop 1978; Jobling 1982; Braaten 1984; Holdway and Beamish 1984; Hawkins et al. 1985; Kjesbu et al. 1991).

Several factors can explain the lower growth rates observed during the autumn experiment and the difference in the pattern of response in growth rate with salinity between the two experiments (Fig. 1). Season (spring versus autumn), food item and energetic value (smelt versus capelin), and size and maturational state of cod all differed between the experiments. Cod used in the spring experiment were significantly smaller than those in the autumn experiment (Table 1). The GSI of cod at the beginning of the spring experiment was low and characteristic of nonmaturing fish and remained constant throughout the experiment. On the contrary, cod used in the autumn experiment showed a significant development of their gonads during the course of the experiment, with GSI values increasing from 0.7 to 1.1% at the beginning of the experiment to values of 3.4–6.6% at the end of the experiment (Table 2). Size is largely known to influence growth rate of fish. Specific growth rates obtained in this study were compared with specific growth rates found in the literature for cod of similar size in growth experiments conducted at 10°C. Based on several studies carried out at various temperatures and on different sizes of cod, Jobling (1988) established a relationship describing the effects of temperature and size on growth rate for cod with feeding regimes that were assumed to promote maximum rates of growth (Jobling 1988). Examination of specific growth rate (natural logarithm) as a function of mean weight of fish (natural logarithm) for cod fed three times weekly shows a very good correspondance between observed growth rates in seawater (28‰) for both spring and autumn experiments and predicted growth rates calculated from the equation of Jobling (1988) (Fig. 2). More interestingly, these data clearly demonstrate that specific growth rates obtained in lower salinities (7 and 14‰) are higher than mean growth rates obtained in the other studies reported by Jobling (1988) in full salinity conditions (32–34‰). Another conclusion that can be drawn from the examination of the relationship between specific growth rate and weight of fish (Fig. 2) is that differences in growth rate between the spring and autumn experiments for salinity conditions of 14 and 28‰ are largely associated with differences in the mean weight of fish between the two experiments. However, growth rate of cod in the autumn experiment in a salinity of 7‰ is lower than what should be expected on the basis of weight differences

only (Fig. 2). Maturation of cod near its physiological limit of salinity tolerance (Provencher et al. 1993) during the autumn experiment could have contributed to decreasing the growth rate at 7‰. However, osmotic concentrations measured on subsamples of fish during the autumn experiment for the low salinity condition (7‰) were well within the range of values observed by Dutil et al. (1992) and for which there were no indications that acclimation was stressful for cod. Cod maintained in 7‰ salinity during the autumn experiment still showed growth rates comparable with those observed in seawater (28‰).

Effects of Salinity on Food Intake and Food Conversion Efficiency

Differences in growth rates with salinity are closely associated with differences in food conversion efficiencies between salinities (Fig. 1). While food intake is little affected by salinity, differences in food conversion efficiencies between salinities follow a pattern that clearly reflects the growth response of cod to salinity (Fig. 1). The lower food intake observed for cod at a salinity of 7‰ during the autumn experiment did not result in significantly lower growth rate at 7‰ compared with 28‰ (Fig. 1). Thus, it appears that lower food intake would have been needed to result in lower growth at 7‰. Many studies demonstrated that salinity influences food intake and food conversion efficiency (Otto 1971; Brocksen and Cole 1972; MacLeod 1977; Arunachalam and Reddy 1979; Kilambi 1980; Gutt 1985; Dendrinos and Thorpe 1985; McCormick et al. 1989). However, responses to salinity differed depending on species, ration, and temperature. Food intake of presmolt coho salmon (*Oncorhynchus kisutch*) has been shown to decrease at higher salinities (Otto 1971), while rainbow trout (*Oncorhynchus mykiss*), freshwater catfish (*Mystus vittatus*), European bass (*Dicentrarchus labrax*), and juvenile European flounder have shown to increase their food intake at intermediate or higher salinities (MacLeod 1977; Arunachalam and Reddy 1979; Dendrinos and Thorpe 1985; Gutt 1985). These same studies also demonstrated that coho salmon, rainbow trout, and freshwater catfish had higher food conversion efficiencies in freshwater and low salinity conditions whereas European bass and juvenile European flounder reached their highest food conversion efficiencies in intermediate salinity conditions. McCormick et al. (1989), on the other hand, did not observe any effect of salinity on food conversion efficiency for Atlantic salmon (*Salmo salar*) smolts.

Effects of Salinity on Metabolism, Digestion, and Absorption

Higher growth rates and better food conversion efficiencies at intermediate salinities in this study may have resulted from a reduction in the metabolic demand and from more efficient digestive and absorptive processes. Many studies on the effects of salinity on fish have been centered on the energy demands of osmotic and ionic regulation. The usual hypothesis proposed is that the energetic cost of ion regulation is lowest in an isosmotic environment, where the ionic gradients between blood and water are minimal, and that this energy saving is substantial enough to increase growth (Morgan and Iwama 1991). However, many studies reported by Morgan and Iwama (1991) failed to show that maximal growth rates were obtained in an isosmotic environment. Moreover, a large variability in the estimates of the energetic cost of osmoregulation is found in the literature, with estimated values varying between 1 and 40% of the resting metabolism (Rao 1968; Eddy 1982; Pérez-Pinzón and Lutz 1991). Changes in salinity will also modify drinking rates (Smith 1930; Conte 1969) and could consequently affect stomach evacuation rate, rate of food movement in the intestine, absorption efficiency, pH of the gastric fluid, and food breakdown (DeSilva and Perera 1976; MacLeod 1977; Ferraris et al. 1986). Further experiments designed to measure standard metabolic rate and food absorption efficiency of Atlantic cod in different salinity conditions are required to show how growth rate and food conversion efficiency are affected by changes in salinity.

Like the vast majority of marine species, Atlantic cod are mainly distributed in oceanic waters with salinities around 34‰. Populations living in brackish waters, such as occur in the Baltic, or juvenile stages living in river estuaries or near surface in coastal areas may benefit from lower salinities provided that the productivity of low salinity waters is high. In the context of cod aquaculture, rearing cod at intermediate salinities, such as in estuaries or in land-based facilities provided with brackish groundwater at constant temperature, would confer a marked advantage compared with water at 28‰ and even more so compared with water at 34‰.

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Comparison of Toxic Equivalent Factors for Selected Dioxin and Furan Congeners Derived Using Fish and Mammalian Liver Cell Lines

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Clemons, J.H., M.R. van den Heuvel, J.J. Stegeman, D.G. Dixon, and N.C. Bols. 1994. Comparison of toxic equivalent factors for selected dioxin and furan congeners derived using fish and mammalian liver cell lines. Can. J. Fish. Aquat. Sci. 51: 1577–1584.

Toxic equivalent factors (TEFs) for eight polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) congeners were derived with a rainbow trout (*Oncorhynchus mykiss*) cell line, RTL-W1, and compared with TEFs obtained with a rat hepatoma cell line, H4IIE. Cells were exposed to a range of concentrations of the congeners which included 1,2,3,7,8-pentaCDD, 1,2,3,4,7,8-hexaCDD, 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, and 1,2,3,4,7,8-hexaCDF. Ethoxyresorufin *o*-deethylase (EROD) activity was measured and EC50 values calculated from a dose–effect curve newly proposed for this purpose. TEFs were computed using 2,3,7,8-tetraCDD standard curves run concurrently with each assay. With the exception of 1,2,3,6,7,8-hexaCDD and 1,2,3,7,8-pentaCDF, all of the RTL-W1-derived TEFs were significantly higher (two- to eightfold higher) than the respective H4IIE TEFs. Immunoblotting analysis with the monoclonal anti-scup P4501A1 antibody was used to identify basal and induced levels of P4501A1 protein in both the RTL-W1 and H4IIE cells. It was concluded that mammalian-derived TEFs may not accurately predict the potency of PCDDs or PCDFs to fish.

Nous avons calculé les facteurs d'équivalence de toxicité (FÉT) pour huit congénères de la dibenzo-*p*-dioxine (PCDD) et du dibenzofurane polychloré (PCDF) dans une ligné cellulaire de la truite arc-en-ciel (*Oncorhynchus mykiss*), RTL-W1, et les avons comparés aux FÉT obtenus avec une lignée cellulaire de l'hépatome du rat, H4IIE. Les cellules ont été exposées à une gamme de concentrations des congénères, lesquels incluaient : 1,2,3,7,8-pentaCDD, 1,2,3,4,7,8-hexaCDD, 1,2,3,6,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, 2,3,7,8-tétraCDF, 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF et 1,2,3,4,7,8-hexaCDF. L'activité de l'éthoxyrésorufine *o*-déséthylase (EROD) a été mesurée et les valeurs de CE50 ont été calculées à partir d'une courbe dose–effet récemment proposée à cette fin. Les FÉT ont été calculés à partir de courbes d'étalonnage pour la 2,3,7,8-tétraCDD, effectuées en parallèle lors de chaque épreuve. À l'exception de la 1,2,3,6,7,8-hexaCDD et du 1,2,3,7,8-pentaCDF, tous les FÉT calculés à partir des cellules RTL-W1 sont significativement supérieurs (de deux à huit fois) aux FÉT respectifs obtenus dans les cellules H4IIE. L'analyse par immunotransfert avec l'anticorps monoclonal P4501A1 a été utilisée pour déterminer les concentrations basales et induites de la protéine P4501A1 dans les cellules RTL-W1 et H4IIE. Nous concluons que les FÉT obtenus à partir des cellules mammaliennes ne permettent pas nécessairement de prédire avec exactitude la toxicité des PCDD ou des PCDF chez les poisson.

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Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), chemicals classified as polyaromatic halogenated hydrocarbons (PHHs), are environmentally ubiquitous, persistent, and bioaccumulative in aquatic organisms. As there are 75 dioxin and 135 furan congeners, all with different toxic potencies, the assessment of the environmental impact of these congeners and their mixtures is difficult. The compounds with the highest toxicity and persistence are substituted with chlorine atoms in the 2,3,7,8 ring positions. In both fish and mammals, the 2,3,7,8-substituted dioxin and furan congeners strongly induce expression of a cytochrome P450 gene, CYP1A1. The protein product, P4501A1, can be measured either by immunochemical analysis with anti-P4501A1 antibodies or by catalytic assays such as that for ethoxyresorufin *o*-deethylase (EROD).

The toxicity of these structurally similar congeners is thought to be mediated through the binding of the chemical to a cytosolic receptor, the aryl hydrocarbon (Ah) receptor,

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resulting in the activation and translocation of the receptor–ligand complex to the nucleus where transcription of different genes, including P4501A1, is induced. The magnitude of the in vivo induction of P4501A1 in rats correlates well with other toxic endpoints such as thymic atrophy, body weight loss, and immunosuppression, implying that these endpoints are all mediated through the Ah receptor (Safe 1990). Evaluation of the relative toxic potency of PHHs has commonly been achieved through the comparison of the ability of these compounds to induce P4501A1 (Mason et al. 1986; Safe 1990). This has led to the development of in vitro bioassays that provide fast and efficient means of ranking chemical toxicity. Chemicals are ranked relative to the most potent congener, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-tetraCDD). The resulting potency ratio is called a toxic equivalent factor (TEF).

Determination of TEFs for PHHs has been mainly based on mammalian assays to provide potency ratios for human risk assessment. The H4IIE rat hepatoma cell line bioassay for P4501A1 induction has been widely used to this end (Bradlaw and Casterline 1979; Mason et al. 1986; Tillitt et al. 1991). Because recent work has shown significant differences in TEFs for fish relative to mammals (Walker and Peterson 1991; Tillitt and Cantrell 1992; Parrott et al. 1993), the accuracy of extrapolating mammalian values to fish has been questioned. Consequently, determination of the relative potency of these compounds specifically for fish is desirable. In research analogous to the determination of mammalian TEFs with the H4IIE cell line, the use of both primary cell cultures and cell lines from fish for the resolution of congener-specific PHH potency has been initiated (Pesonen et al. 1992; Tillitt and Cantrell 1992).

Lee et al. (1993) recently described a rainbow trout (*Oncorhynchus mykiss*) liver cell line, RTL-W1, that contains 2,3,7,8-tetraCDD-inducible EROD activity. We used this cell line to determine TEFs for four dioxin and five furan congeners, based on the induction of P4501A1, as determined by EROD activity. The P4501A1 protein was identified by immunoblot analysis with anti-scup (*Stenotomus chrysops*) P4501A1 (previously called P450E) mouse monoclonal antibody 1-12-3 (Mab 1-12-3). The RTL-W1 TEFs were compared with H4IIE TEFs, also derived in our laboratory, in order to test the null hypothesis that there is no difference between the relative potency of the nine congeners to fish and mammalian cell lines. To date, little attention has been devoted to the evaluation of methods of determining EC50s (the congener concentration causing a 50% of maximal EROD activity) for dose–effect curves or to the statistical uncertainty of bioassay-derived TEFs. Confrontation of these problems is needed for this in vitro bioassay to become a more widely accepted bioanalytical tool. Consequently, two methods of deriving EC50s were compared and the variability associated with the TEF estimates examined.

Methods

Experimental Design

Mammalian (H4IIE) and fish (RTL-W1) cells were exposed to six graded doses of the nine 2,3,7,8-substituted dioxin and furan congeners tested. Two curve-fitting methods for determining EC50s were compared and the most suitable was selected. The dose–effect curves were used to generated EC50s for each congener. The ratio of the 2,3,7,8-tetraCDD EC50 to the congener EC50 was used to derive TEFs for the comparison of the potency of these chemicals with respect to their ability to induce P4501A1 in the two cell lines. Immunochemical analysis was performed with Mab 1-12-3 to verify the enzyme induced as P4501A1.

Chemicals

All dioxin congeners (2,3,7,8-tetraCDD, 1,2,3,7,8-pentaCDD, 1,2,3,4,7,8-hexaCDD, 1,2,3,6,7,8-hexaCDD, and 1,2,3,4,6,7,8-heptaCDD) and three furan congeners (1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, and 1,2,3,4,7,8-hexaCDF) were received from the Canadian Centre for Inland Waters (CCIW), Burlington, Ont., and originated from Cambridge Isotope Laboratories (Woburn, Mass.). The 2,3,7,8-tetraCDF was purchased directly from Cambridge Isotope Laboratories. The concentrations and purity of the stock solutions in isooctane were measured by gas-chromatography/mass-spectroscopy at CCIW. All standards were found to be greater than 99.5% pure; measured concentrations were used when determining the TEFs. Chemicals were transferred to the bioassay carrier solvent dimethyl sulphoxide (DMSO) by nitrogen evaporation of the isooctane phase; the concentrations required for the assays were prepared by serial dilution.

Cell Lines and Culturing Techniques

RTL-W1

The rainbow trout liver cell line, RTL-W1 (Lee et al. 1993), was maintained in Leibovitz's L-15 medium (Gibco BRL, Burlington, Ont.). The medium was supplemented with 5% fetal bovine serum (FBS, GIBCO BRL) and penicillin–streptomycin (100 U penicillin, 100 μg streptomycin·mL^{-1}, ICN/Flow, Mississauga, Ont.). Cells were grown in 75-cm^2 Nunc tissue-culture flasks and maintained at 22 ± 1°C.

H4IIE

The rat hepatoma cell line (H4IIE) was purchased from the American Type Culture Collection (ATCC No. CRL 1548). Medium and maintenance conditions were similar to those specified by Tillitt et al. (1991). Dulbecco's modified Eagle's medium (MEM, Sigma, St Louis, Mo.) was used for culturing H4IIE. Supplements to the media included L-glutamine (1×, Sigma), gentamycin sulphate (0.05 mg·mL^{-1}, Sigma), MEM essential amino acids (1.5×, Sigma), MEM nonessential amino acids (2×, Sigma), MEM vitamins (1.5×, Sigma), and 15% FBS (heat inactivated, Sigma). The cell cultures were grown in 75-cm^2 Costar tissue-culture flasks in a humidified CO_2 (5%) atmosphere at 37 ± 1°C. A repository of cells was frozen two passages after receipt from the ATCC. Cells were not used for more than 10 passages.

Bioassay Procedure

Originally the isooctane carrier of Tillitt et al. (1991) was used in this study. DMSO was substituted because isooctane was not soluble in the culture media and was cytotoxic to RTL-W1. Dose–effect curves and EC50 values obtained with DMSO and isooctane were compared to determine if there was a carrier effect on EROD activity. In addition, the relative effect of the two carriers on the distribution of [^{3}H]2,3,7,8-tetraCDD (Cambridge Isotope Laboratories) was determined to the H4IIE system. After 48 h of exposure to [^{3}H]2,3,7,8-tetraCDD, both the medium and cell homogenate

were extracted with toluene and the activity determined by liquid scintillation counting.

RTL-W1

Approximately 5×10^5 cells were seeded into 15 × 60 mm cell culture dishes in a 5 mL of L-15 medium and allowed to grow for 3–5 d. Upon confluency, the medium was changed and either the DMSO carrier (solvent control) or the appropriate amount of the inducing agent was introduced. The cells were never exposed to more than 0.5% DMSO, a concentration we found not to be cytotoxic. After a 72-h exposure to the chemical, the EROD assay was completed as outlined below. Each bioassay used a minimum of six concentrations of the inducing agent. A 2,3,7,8-tetraCDD standard curve was run concurrently with the test congener in each assay. The bioassays were replicated a minimum of three times.

H4IIE

Procedures were similar to those for RTL-W1. The cells were seeded in 15 × 60 mm culture dishes at a density of 1.0×10^6 cells per plate in 5 mL of medium. After 24 h cells were exposed to either DMSO carrier or the inducing chemical. The EROD assays were completed after a 48-h exposure because no significant differences in EC50 values were observed for cells assayed at 48 versus 72 h.

EROD Assay

The EROD assay was performed as described by Tillitt et al. (1991) with some modifications. The medium was aspirated from the cells followed by a rinse with phosphate-buffered saline (pH 7.8). The cells were then scraped from the plate in Tris–sucrose buffer (0.05 mM Tris, 0.2 M sucrose, pH 7.8). The supernatant was aspirated from the pelleted cells after a refrigerated 650 × *g* centrifugation spin. An aliquot of the Tris–sucrose buffer (2 mL for H4IIE, 0.5 mL for RTL-W1) was added to the cell pellet, prior to sonication. Sonication facilitated the quantification of protein but had no effect on EROD activity.

In order to determine EROD activity, a protein aliquot of either 200 μL (H4IIE) or 150 μL (RTL-W1) was added to the reaction mixture that contained 1 mL of HEPES buffer (0.1 M HEPES, pH 7.8), 10 μL of $MgSO_4$ (5.3 mM), 50 μL of bovine serum albumin V (40 mg BSA·mL^{-1}, Sigma), and 20 μL of NADPH (0.54 mM, Boehringer Mannheim, Laval, Que.). The reaction, incubated at 25°C for RTL-W1 and 37°C for H4IIE, was started with the addition of 10 μL of 7-ethoxyresorufin in DMSO (7-ER, 1.5 μM in final reaction mixture, Sigma) and stopped after 10 min with 2 mL of cold methanol. The protein precipitate was allowed to settle for 10–15 min before centrifugation at 650 × *g* (20°C) for 30 min. The resorufin product was quantified using a Turner 430 spectrofluorometer with 550-nm excitation (15-nm slit width) and 585-nm emission (60-nm slit width) wavelengths. The final EROD activity was expressed in picomoles of resorufin per milligram of protein (P) per minute of reaction time.

Protein levels were determined using the method of Bradford (1976) where 5 μL of the cell suspension was added to 250 μL of the Bio-Rad solution (four volumes of deionized water to one volume of Bio-Rad dye agent, Bio-Rad, Mississauga, Ont.) in triplicate wells within a 96-well plate. The absorbance was read on an Elisa Immunoassay (EIA) microplate reader using a 590-nm filter. Protein concentrations were determined relative to a BSA standard curve.

Curve-Fitting Method Comparison

Two curve-fitting methods for the estimation of EC50 values were compared using data from the 2,3,7,8-tetraCDD standard dose–effect curves derived with the H4IIE cell line. The first method was derived from a receptor-based kinetics model (O'Flaherty 1981) as

(1) $$\text{EROD activity} = \text{background activity} + \frac{\text{EROD}_{\max} \times \text{DOSE}}{\text{EC50} + \text{DOSE}}.$$

EC50 estimations were based on a logit function for continuous response data (Hosmer and Lemeshow 1989) as follows:

(2) $$\text{EROD activity} = \text{background activity} + \frac{(\text{EROD}_{\max} - \text{background activity})}{1 + e^{\beta - (\gamma \times \ln \text{dose})}}$$

where $\text{EROD}_{\max}$ is the maximum level of EROD induction, background activity is the activity of the solvent control, β is the location parameter for the curve, and γ is the slope parameter for the curve. Through a manipulation of Eq. 2, the EC50 can be calculated as

(3) $$\text{EC50} = e^{\frac{\beta}{\gamma}}.$$

Toxic Equivalent Factors

TEFs expressing the inductive potency of each congener relative to 2,3,7,8-tetraCDD, expressed on a molar basis, were calculated after Kutz et al. (1990) as

(4) $$\text{TEF} = \frac{\text{EC50}_{\text{TCDD}}}{\text{EC50}_{\text{CONGENER}}}.$$

A minimum of four assays was run to determine individual TEFs for each chemical. Mean (SE) TEFs were derived for the eight congeners.

Immunochemical Analysis

Immunochemical analyses were performed in order to verify that the enzyme being measured in the EROD assay was P4501A1. The PHH-inducible trout protein, previously called $P450LM_4$, is designated P4501A1 because of homology of the trout cDNA sequence to the P4501A1 protein (Heilmann et al. 1988). Both the rat and fish cell lines were exposed to either 2,3,7,8-tetraCDD or DMSO. 2,3,7,8-TetraCDD exposure concentrations (390 pM for H4IIE, 97.5 pM for RTL-W1) were chosen to give maximal EROD response. Three sets of duplicate plates were exposed; cells from plate pairs were pooled to achieve a triplicate design. The cells were sonicated after being rinsed and spun down as described for the bioassay procedure. One 450-μL aliquot was used in the EROD assay, while a second was frozen with dry ice in 4× the volume of freezing buffer (0.1 M PO_4, 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol, pH 7.5) and stored in liquid nitrogen for immunochemical analysis.

The preparation of cells for immunoblotting was as described by Eberhart et al. (1992). The frozen cells were thawed and resuspended in cold (4°C) buffer (0.05 M Tris,

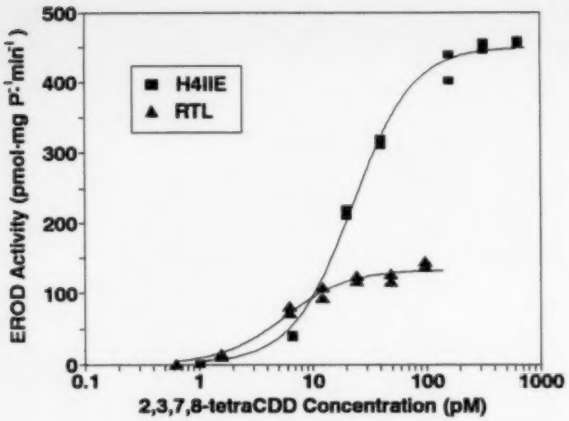

FIG. 1. Representative EROD dose–effect curves for RTL-W1 and H4IIE after induction with six consecutive 2,3,7,8-tetraCDD doses. EROD induction was measured after 72 h for RTL-W1 and 48 h for H4IIE.

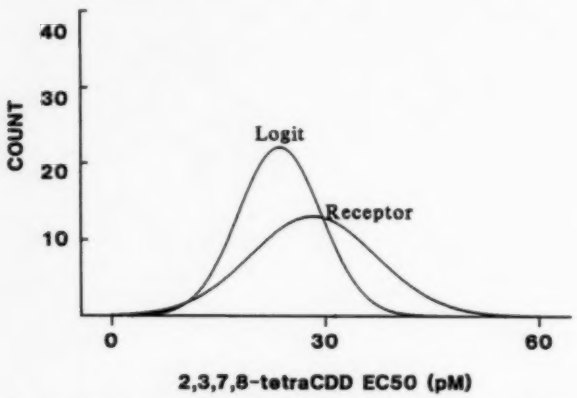

FIG. 2. Normal distributions of EC50 values determined with either receptor- or logit-based curve-fitting techniques. The data represent the results of 79 replicate 2,3,7,8-tetraCDD dose–effect curves with the H4IIE cell line.

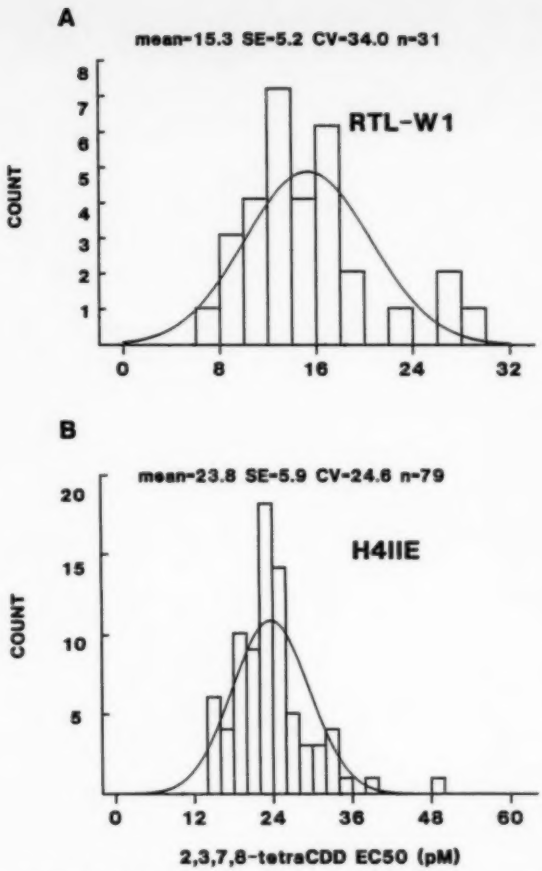

FIG. 3. Distribution of EC50 values from EROD dose–effect curves obtained with 2,3,7,8-tetraCDD-induced (A) RTL-W1 and (B) H4IIE cells over the course of the study.

0.15 M KCl, pH 7.4) and homogenized (on ice) with 10 passes of a Teflon-coated pestle in a glass tube-mortar. This homogenate was centrifuged at 750 × *g* (10 min) and 12 000 × *g* (10 min) to obtain the supernatant for a 70-min microsomal extraction at 100 000 × *g*. The pelleted microsomes were resuspended in 0.5–1.0 mL of buffer (0.05 M Tris, 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol, pH 7.5).

Mab 1-12-3 was used for immunoblotting. Mab 1-12-3 is specific for both the rainbow trout P4501A1 and the rat P4501A1 enzyme with little specificity for the P4501A2 enzyme also present in the H4IIE cell line (Goksoyr et al. 1991). Thus, this antibody was appropriate for identifying the enzyme responsible for induction by dioxin in both cell lines. The immunoblotting procedure was done with microsomal fractions as described in Kloepper-Sams et al. (1987), using goat anti-mouse IgG linked to alkaline phosphatase (Bio-Rad); staining used nitroblue tetrazolium and 5-bromo-4-chloro-3-indoylphosphate. To account for P4501A2 induction, a polyclonal anti-rabbit P4501A2 (Oxford Biomedical Research, Inc) was used in place of the Mab 1-12-3. The amount of stain was quantified by video imaging densitometry (Master Scan, Scanyltics/CSPI, Billerica, Mass.).

Statistics

All statistics were performed using SYSTAT software (Wilkinson 1990). Mean and standard error were calculated within the STATS module. The curve-fitting program was through a basic MACRO written in SYSTAT that utilized the NONLIN module of SYSTAT. A *t*-test for independent means was run in SYSTAT for all mean comparisons (solvent carrier data, TEFs, immunoblotting analysis). Pearson correlations testing for probability of fit and significance were performed in the CORR module for SYSTAT. For all statistical analysis, the critical level of significance was $\alpha = 0.05$.

Results

P4501A1 Induction and Curve-Fitting Comparison

For both the RTL-W1 and H4IIE cell lines, EROD activity showed a sigmoidal dose–effect curve to 2,3,7,8-tetraCDD (Fig. 1). The two models for deriving EC50 values were compared using H4IIE 2,3,7,8-tetraCDD dose–effect data (Fig. 2). The mean EC50s for both methods of curve-fitting

TABLE 1. Mean EC50s (pM) (SE, CV (%), and n in parentheses) of EROD induction for nine PCDD and PCDF congeners determined with the RTL-W1 and H4IIE cell lines.

Congener	RTL-W1	H4IIE
Dioxins		
2,3,7,8-tetraCDD	15.3 (5.2, 34.0, 31)	23.8 (5.9, 24.7, 79)
1,2,3,7,8-pentaCDD	6.0 (1.1, 18.3, 5)	21.7 (6.8, 31.3, 4)
1,2,3,4,7,8-hexaCDD	11.5 (1.6, 13.9, 4)	56.4 (4.7, 8.3, 4)
1,2,3,6,7,8-hexaCDD	97.5 (16.5, 16.9, 4)	150.9 (37.5, 24.9, 4)
1,2,3,4,6,7,8-heptaCDD	154.8 (30.2, 19.5, 3)	271.2 (65.4, 24.1, 4)
Furans		
2,3,7,8-tetraCDF	71.1 (37.2, 52.3, 4)	923.4 (464.7, 50,3, 6)
1,2,3,7,8-pentaCDF	101.9 (32.8, 32.2, 4)	218.8 (51.6, 23.6, 4)
2,3,4,7,8-pentaCDF	8.2 (2.6, 31.7, 4)	28.9 (13.3, 46.0, 4)
1,2,3,4,7,8-hexaCDF	20.1 (3.5, 17.4, 4)	119.0 (37.0, 31.1, 4)

were not statistically different, but the coefficient of variation (CV) was larger with the receptor-based equation (CV = 31.4%) than with the logit equation (CV = 24.6%). For this reason, and because the receptor-based equation overestimated the response at lower doses, the logit technique was considered descriptively superior and was used to generate EC50s for TEF analysis with both H4IIE and RTL-W1 for the remainder of the study.

The mean EC50 values for H4IIE exposed to 2,3,7,8-tetraCDD using DMSO as the carrier was 15.31 ± 5.21 pM (n = 79) and isooctane as a carrier was 13.19 ± 4.41 pM (n = 4). The maximum EROD activity was 413.96 ± 26.44 and 147.76 ± 49.67 pmol·mg protein^{-1}·min^{-1} for DMSO and isooctane carriers, respectively. Therefore, although there was no effect of carrier on the EC50 for EROD induction by 2,3,7,8-tetraCDD in the H4IIE cell line, the maximum EROD activity was approximately fourfold higher with DMSO. Experiments with [^{3}H]2,3,7,8-tetraCDD in isooctane or DMSO indicated that approximately 50% of the measurable radioactivity remained in the media, while 50% was associated with the cellular fraction, regardless of which carrier was used.

For both cell lines, the derived EC50 values for EROD induction by 2,3,7,8-tetraCDD obtained were normally distributed (Fig. 3). The mean (SE, n) 2,3,7,8-tetraCDD EC50 values for the RTL-W1 and H4IIE cell lines were 15.3 (5.2, 31) and 23.8 (5.9, 79) pmol·mg protein^{-1}·min^{-1}, respectively. Based on the 2,3,7,8-tetraCDD EC50s, the H4IIE cell line (CV = 24.6%) showed less day-to-day variability than the RTL-W1 line (CV = 34.0%). The mean CV ± SE for the EC50s generated for all of the congeners was 29.4 ± 4.2% for H4IIE and 26.2 ± 4.13% for RTL-W1 (Table 1).

In RTL-W1 and, to a lesser degree, H4IIE, the maximum EROD activity achieved with some congeners (1,2,3,6,7,8-hexaCDD and 1,2,3,4,6,7,8-heptaCDD) was significantly less than the maximum reached with concurrently run 2,3,7,8-tetraCDD standards, and the slope of the dose–effect curves did not always appear parallel to that of 2,3,7,8-tetraCDD (Fig. 4). Despite this, the EC50s for these congeners were very consistent.

Immunochemical Analysis

Immunoblotting with Mab 1-12-3 detected P4501A1 protein in both the RTL-W1 and the H4IIE cells (Fig. 5). Levels of P4501A1 between RTL-W1 and H4IIE cannot be compared directly because the relative affinity of the monoclonal antibody for P4501A1 for the trout and rat is unknown. Yet, the H4IIE cells consistently showed higher levels of both P4501A1 and EROD activity than the RTL-W1 relative to DMSO controls. For RTL-W1, 2,3,7,8-tetraCDD-exposed cells showed respective 4- and 77-fold increases in P4501A1 levels and EROD activity relative to the DMSO controls. H4IIE cells showed respective increases of 11- and 322-fold for P4501A1 levels and EROD activity.

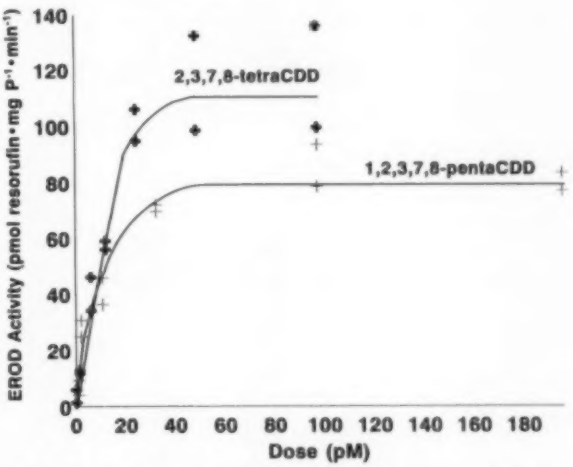

FIG. 4. Representative EROD dose–effect curves for RTL-W1 of 2,3,7,8-tetraCDD and 1,2,3,7,8-pentaCDD run concomitantly. This figure shows that the slopes and the achieved maximum induction can differ between congeners.

Toxic Equivalent Factors

Table 2 summarizes the TEFs for the dioxins and furans with both cell lines during this study. With standard curves run with each assay, the CV of TEF values averaged 23.1 ± 1.9 and 23.5 ± 3.1% for H4IIE and RTL-W1, respectively. The RTL-W1 cells had TEFs significantly greater than 1.0 for two of the eight congeners (1,2,3,7,8-pentaCDD and 2,3,4,7,8-pentaCDF). The values for congeners 1,2,3,4,7,8-hexaCDD and 1,2,3,4,7,8-hexaCDF for RTL-W1 and 1,2,3,7,8-pentaCDD for H4IIE were not different from 1.0. All other congeners had average TEFs less than 1.0. For both cell

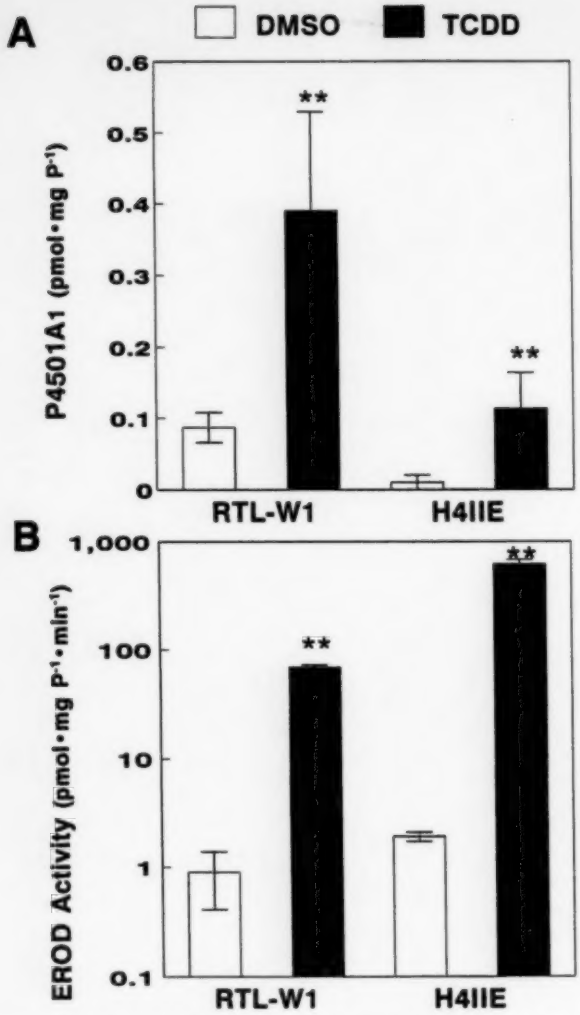

FIG. 5. Levels of P4501A1 by (A) immunoblot analysis and (B) EROD activity shown by the RTL-W1 and H4IIE cell lines. The cells were exposed to 97.5 (RTL-W1) or 390 (H4IIE) pM of 2,3,7,8-tetraCDD; carrier controls (DMSO) were also completed for both cell lines. Values are given as means ± SE, all $n = 3$. In all cases, the treated cells showed significantly ($p < 0.05$) higher levels of activity than their respective DMSO controls.

lines, the inductive potency of the dioxins ranked from most to least potent was 1,2,3,7,8-pentaCDD, 1,2,3,4,7,8-hexaCDD, 1,2,3,4,6,7,8-heptaCDD, and 1,2,3,6,7,8-hexaCDD. For furans, the most potent congener was 2,3,4,7,8-pentaCDF, followed by 1,2,3,4,7,8-hexaCDF, 1,2,3,7,8-pentaCDF, and 2,3,7,8-tetraCDF.

The mean TEFs derived for RTL-W1 and H4IIE were compared for each congener in order to determine differences in sensitivity. With the exception of 2,3,7,8-tetraCDF, the order of potency was similar between RTL-W1 and H4IIE cells. In six of the eight cases, the TEF was higher for RTL-W1 relative to H4IIE. Compared with H4IIE TEFs, RTL-W1 TEFs were on average 2.2-fold higher for 1,2,3,7,8-pentaCDD, 1,2,3,4,7,8-hexaCDD, and 1,2,3,4,6,7,8-heptaCDD, 4.2-fold higher for the furan congeners 2,3,4,7,8-pentaCDF, 1,2,3,7,8-hexaCDF, and 2,3,7,8-tetraCDF TEFs for RTL-W1, and 6.7-fold greater for 2,3,7,8-tetraCDF.

The RTL-W1 and H4IIE TEFs correlated well with TEFs generated in the study by Walker and Peterson (1991). For the congeners 2,3,4,7,8-pentaCDD, 1,2,3,4,7,8-hexaCDD, 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, 2,3,4,7,8-pentaCDF, and 1,2,3,4,7,8-hexaCDF, the early life stage mortality TEFs correlated significantly with both RTL-W1 ($r^2 = 0.967$, $p = 0.002$) and H4IIE ($r^2 = 0.963$, $p = 0.002$) TEFs.

Discussion

This work demonstrated that RTL-W1 has inducible P4501A1 and has potential to be used as a rapid and effective tool for establishing international toxic equivalent factors (I-TEFs) for fish. The dose–effect curves with RTL-W1 were repeatable and showed levels of variability comparable with those seen with the rat hepatoma cell line H4IIE. TEFs were derived using EC50s from these curves. By this criterion and for the congeners tested, RTL-W1 was more sensitive.

Two different curve-fitting methods were analyzed and compared using the H4IIE dose–effect data for 2,3,7,8-tetraCDD. The use of the logit function provided a better description of the relationship than the receptor-based function and resulted in decreased variability in the estimated parameters. Although the receptor-based equation was derived from known theory of receptor binding and expression of the P4501A1 enzyme and should be fundamentally correct, it appeared to overestimate the average maximum enzyme activity. This method also overestimated the activity at low doses seen clearly in the dose–effect curves generated. These discrepancies were not entirely surprising, as the relationship does not take temporal effects into account. At low doses of 2,3,7,8-tetraCDD, expression of P4501A1 peaked at 24 h (authors, unpublished data) and was declining at the time that the assays were performed (48 h). Although the logit function is a strictly empirical approach, it is descriptively superior to the theoretical relationship. Both methods used here use nonlinear curve-fitting techniques that determine an average value for maximal enzyme induction. This approach is preferred to previous methods that required the choice of maximum level of activity before a relationship is applied to the data.

Immunoblot analysis demonstrated background and induced levels of P4501A1 in both cell lines. The Mab 1-12-3 antibody has been shown to be very specific for the P4501A1 of many fish species including rainbow trout (Stegeman 1989; Goksoyr et al. 1991), but less reactive to rat P4501A1 (Goksoyr et al. 1991). Because of the differential affinity of Mab 1-12-3 for trout versus rat P4501A1, a direct comparison of P4501A1 protein levels between species was not possible. One obvious difference between the two cell lines is the maximum level of EROD induction between the H4IIE and RTL-W1. A second difference between the two cell lines involves the variability of the EC50s obtained with each. The CV for the RTL-W1 2,3,7,8-tetraCDD EC50s was consistently higher than for comparable H4IIE TEFs. The difference may be accounted for by the lower amount of total protein, compared with that produced by H4IIE which results in less sensitive measurement. The error in estimated TEFs for the 2,3,7,8-tetraCDD standard EC50s was partially mitigated by completing a standard curve with each

TABLE 2. Mean TEFs (SE, CV, and n in parentheses) derived with either RTL-W1 or H4IIE cell lines for selected polychlorinated dibenzo-p-dioxins and dibenzofurans. I-TEFs (Kutz et al. 1990; Safe 1990) and rainbow trout early life stage mortality (Walker and Peterson 1991) are also listed for comparison. For each congener, the RTL-W1 and H4IIE TEFs were significantly different ($p < 0.05$) except where indicated with an asterisk.

Congener	RTL-W1	H4IIE	Rainbow trout early life stage mortality[a]	I-TEF
Dioxins				
2,3,7,8-tetraCDD	1.0	1.0	1.0	1.0
1,2,3,7,8-pentaCDD	2.6 (0.7, 26.9, 5)	1.1 (0.3, 27.3, 4)	0.7	0.5
1,2,3,4,7,8-hexaCDD	1.1 (0.3, 27.3, 4)	0.5 (0.08, 16.0, 4)	0.3	0.1
1,2,3,6,7,8-hexaCDD*	0.2 (0.05, 25.0, 5)	0.2 (0.04, 20.0, 4)		0.1
1,2,3,4,6,7,8-heptaCDD	0.2 (0.04, 20.0, 3)	0.1 (0.02, 20.0, 4)		0.01
Furans				
2,3,7,8-tetraCDF	0.2 (0.01, 5.0, 4)	0.03 (0.01, 33.3, 6)	0.03	0.1
1,2,3,7,8-pentaCDF*	0.2 (0.07, 35.0, 4)	0.2 (0.04, 20.0, 4)	0.03	0.05
2,3,4,7,8-pentaCDF	1.9 (0.4, 21.0, 4)	0.4 (0.1, 25.0, 4)	0.4	0.5
1,2,3,4,7,8-hexaCDF	1.1 (0.3, 27.3, 4)	0.3 (0.07, 23.3, 4)	0.3	0.1

[a]TEFs are defined as 2,3,7,8-tetraCDD LD50 (pmol 2,3,7,8-tetraCDD·g egg^{-1})/congener LD50 (pmol congener·g egg^{-1}) for the same strain of rainbow trout eggs (Walker and Peterson 1991).

assay. The day-to-day variability in the EC50 for the 2,3,7,8-tetraCDD standard may have been due to slight differences in plating density, with a concurrent change in cell response. Regardless of the cause, and consistent with the work of Tillitt et al. (1991), running a standard curve with each assay lowered the CV of the TEFs, relative to those derived with a mean 2,3,7,8-tetraCDD EC50. The TEFs derived with H4IIE by us were similar to those derived with H4IIE by Tillitt et al. (1991) but are different from earlier reports (Bradlaw and Casterline 1979; Mason et al. 1986) that did not use a standard curve with each assay.

There were substantial differences between the TEFs derived with RTL-W1 and H4IIE. For six of the eight PCDD and PCDF congeners tested, RTL-W1 gave significantly higher TEF values than H4IIE. In preliminary results by Tillitt and Cantrell (1992), TEFs compared between a topminnow (*Poeciliopsis lucida*) hepatoma cell line (PLHC-1, Hightower and Renfro 1988) and H4IIE for several PCB congeners were reported as significantly different. Generally, the PLHC-1 line yielded lower TEFs than H4IIE.

Although the basis for these differences is unknown, they could reflect an altered structure of the Ah receptor between fish and mammals. Because the Ah receptor mediates EROD induction, any differences in congener–receptor affinity arising from structural differences in the receptor could account for differences in the TEFs. It is important to note that H4IIE and RTL-W1 each represent only one strain of one species of mammal and fish, respectively, and that the variability in chemical sensitivity within mammalian (Safe 1990) and fish (Walker and Peterson 1991) strains can be significant.

The induction of EROD by dioxins and furans is similar in RTL-W1 and adult rainbow trout in vivo: Parrott et al. (1993) treated rainbow trout orally with the same congeners used in this study and measured EROD activity in their livers. The resulting TEFs correlated well with those reported here for RTL-W1 and H4IIE and in the majority of cases were higher than either H4IIE or international I-TEFs. In addition, a number of congeners including 1,2,3,7,8-pentaCDD and 2,3,4,7,8-pentaCDF yielded TEFs greater than 1.0. It should be noted that Parrott et al.'s TEFs are based on thresholds of induction and not on EC50s as is the case with RTL-W1 and H4IIE.

TEFs derived by Walker and Peterson (1991) for early life stage mortality in rainbow trout are ranked similarly to those calculated with RTL-W1. The correlations between the mortality and RTL-W1 P4501A1 induction are significant. Consequently, this suggests that both endpoints are mediated by a common receptor, the Ah receptor. However, the TEFs derived with RTL-W1 were on average three times higher than those derived with eggs. Only one response, EROD induction, is measured with RTL-W1 whereas early life stage mortality likely measures the accumulation of many different individual responses. Also, whole-organism studies are confounded by the problem of toxicant compartmentalization, and as a result, an administration dose may not accurately reflect the dose at the site of toxic action. Consequently, these differences make direct comparison of TEFs difficult. The higher TEF values with RTL-W1 than with rainbow trout eggs suggest that in order to establish comprehensive piscine TEFs, consideration will have to be given to TEFs derived in different fish systems with different endpoints. For modelling effects on a population level, TEFs generated with larval mortality are probably more appropriate at this time because, as well as differences in absolute values, the in vitro dose–effect curves do not consistently show parallelism and the same level of induction for all congeners. On the other hand, as the underlying causes for differences between the in vitro and in vivo methods become known for parameters such as absolute TEF values and the shapes of the dose–effect curves, the in vitro approach with fish cell lines, such as RTL-W1, promises to be a rapid, inexpensive method of screening large numbers of compounds.

In all cases the RTL-W1 TEFs were greater than the I-TEFs that have been derived from the combined results of several mammalian toxicity tests and applied to environmental risk assessment (Kutz et al. 1990; Safe 1990). The current work suggests that a piscine cell line likely will be more suitable than a mammalian cell line for deriving TEFs to be applied to fish and supports the cautionary note

of Walker and Peterson (1991) about the use of mammalian TEFs for aquatic environment risk assessment. Comparing mammalian (including international) and fish TEFs clearly demonstrates that the use of the former for hazard assessment will underestimate the impact on fish species. However, before RTL-W1 can be used routinely as a bioassay for environmental samples, future research will have to address a number of questions. One is whether known mixtures of dioxins and furans act on RTL-W1 in an additive, synergistic, or antagonistic manner. Another is whether the dose–effect curves that do not parallel the 2,3,7,8-tetraCDD dose–effect curve or reach the same maximal level of induction represent something unique to the congener (in which case a mathematical treatment for dealing with these TEFs will have to be developed) or a methodological problem with the congener. These problems are now being recognized in other systems (Tillitt and Cantrell 1992; Parrott 1993) and are not unique to RTL-W1.

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von Bertalanffy Growth Models with Variability in, and Correlation between, K and L_∞

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A simple set of von Bertalanffy growth models was derived for length at age and for length increment at initial length and time at liberty by assuming that L_∞ and K have the joint bivariate normal distribution with means $\bar{L}_\infty$ and $\bar{K}$, variances $\sigma^2_{L_\infty}$ and σ^2_K, and correlation coefficient ρ, along with two measures of model applicability. Fitting of carapace length increment at initial length and time at liberty of the penaeid *Metapenaeus endeavouri* into the length increment model gave $\bar{L}_\infty = 32.03$ mm, $\sigma^2_{L_\infty} = 1.67$ mm^2, $\rho = -0.44$, $\bar{K} = 2.38 \cdot \text{yr}^{-1}$, and $\sigma^2_K = 0.96 \cdot \text{yr}^{-2}$ with a model error $\sigma^2_e = 0.64$ mm^2, in contrast with $L_\infty = 34.03$ mm, $K = 2.02 \cdot \text{yr}^{-1}$, and $\rho = -0.94$ estimated using the conventional Fabens model. Thus, variability in K was large (41.15%); the negative correlation between L_∞ and K was moderate as against that given in the correlation matrix. Length at age reconstructions indicate that these models would give a good description of growth of *M. endeavouri* within its life span. Sensitivity analysis revealed that its mean length at age and mean length increment for initial length and time at liberty are sensitive to changes in $\bar{L}_\infty$, $\bar{K}$, and σ_K and relatively insensitive to those in σ_{L_∞} and ρ.

Un ensemble de modèles de croissance de von Bertalanffy a été produit pour l'estimation de la longueur en fonction de l'âge et de l'augmentation de la longueur en fonction de la longueur initiale et du moment de la remise en liberté, en supposant que L_∞ et K ont une distribution normale bivariée conjointe ayant pour moyenne $\bar{L}_\infty$ et $\bar{K}$, pour variances $\sigma^2_{L_\infty}$ et σ^2_K et pour coefficient de corrélation ρ, ainsi que deux mesures de l'applicabilité du modèle. L'adaptation de l'augmentation de longueur de la carapace en fonction de la longueur initiale et du moment de la remise en liberté du pénanéide *Metapenaeus endeavouri* au modèle d'augmentation de longueur a permis d'obtenir $\bar{L}_\infty = 32{,}03$ mm, $\sigma^2_{L_\infty} = 1{,}67$ mm^2, $\rho = -0{,}44$, $\bar{K} = 2{,}38 \cdot \text{an}^{-1}$ et $\sigma^2_K = 0{,}96 \cdot \text{an}^{-2}$ et $\sigma^2_e = 0{,}64$ mm^2 pour l'erreur due au modèle, par opposition aux valeurs $L_\infty = 34{,}03$ mm, $K = 2{,}02 \cdot \text{an}^{-1}$ et $\rho = -0{,}94$ estimé à l'aide du modèle classique de Fabens. Ainsi, la variabilité pour K est élevée (41,15 %), la corrélation négative entre L_∞ et K modérée, comme celle donnée dans la matrice de corrélation. Les reconstitutions de la longueur en fonction de l'âge indiquent que ces modèles permettraient de décrire de manière satisfaisante la croissance de *M. endeavouri* durant tout son cycle vital. L'analyse de sensibilité a révélé que sa longueur moyenne en fonction de l'âge et que son augmentation de longueur moyenne en fonction de la longueur initiale et du moment de la remise en liberté sont sensibles au changement des paramètres $\bar{L}_\infty$, $\bar{K}$ et σ_K et relativement insensibles à la variation de σ_{L_∞} et ρ.

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The von Bertalanffy growth equations (VBGEs) are given by

$$(1)\quad l_T = L_\infty[1 - e^{-K(T - T_0)}]$$

for length l_T at age T (von Bertalanffy 1957) and

$$(2)\quad l = (L_\infty - l)(1 - e^{-Kt})$$

for length increment l for initial length l and time at liberty t (Fabens 1965), with parameters K and L_∞ and a time translation T_0. Although derived for a single individual, they have been widely used to model data collected from a group of animals (e.g., Watson and Turnbull 1993). If K and L_∞ vary among individuals in that group, as in most natural populations of animals for genetic reasons, equations 1 and 2 do not generally describe mean length at age, $E[l_T|T]$, or mean length increment for initial length and time at liberty, $E[l|l,t]$. To take individual variability of K and L_∞ into account, Sainsbury (1980) derived analogous VBGEs by assuming L_∞ to be a normal variate and K a gamma variate. In a similar context, Krause et al. (1967) derived a closed form of a probability function for the logistic growth equation with its parameters being treated as independent log-normal variates. The Sainsbury equation for length increment has been used to model data on penaeids (Kirkwood and Somers 1984) and fish (Hampton 1991) and was extended by Smith and McFarlane (1990) to include seasonal growth. A key assumption underlying all those models is independence of their parameters. Yet, both K and L_∞ are generally considered negatively correlated (Gallucci and Quinn 1979; Kingsley 1980; Pauly 1987). Such correlation has been confirmed by Burr (1988) from statistical moments of fish length using minimum cross-entropy inversion and indicated in plots of K against L_∞ estimated from different, but assumed to be comparable, data sets (e.g., Kirkwood and Somers 1984; Watson and Turnbull 1993). It is also interesting to note the obvious but often inappropriate demonstration of correlating K and L_∞ by the plethora of negative correlations generated routinely by some statistical computing packages during fitting VBGEs in the correlation

matrix of estimated parameters (see Discussion). However, the correlation and its implications for the predictability of VBGEs have not been examined.

In this paper, I derive von Bertalanffy growth models for length at age and for length increment for initial length and time at liberty by assuming that K and L_∞ have the joint bivariate normal distribution with a correlation coefficient, examine implications of correlation and variability in both parameters for growth predictions, and finally use these models in a case study to analyse tagging data on the penaeid *Metapenaeus endeavouri*. This bivariate normality can lead to biologically unrealistic negative values of mean length or mean length increment (Sainsbury 1980); two formulae or measures are given for assessing applicability of those models for biological populations. Calculations of both measures will show below that, for *M. endeavouri* data at least, the derived models describe animal growth extremely well within its life span (see Discussion).

Model Formulation

Because of structural similarity between equations 1 and 2, it suffices to derive models for length increment only, which apply immediately to length at age if t is replaced by $T - T_0$ (provided that T_0 is a constant) and l is assigned zero. A joint probability distribution of L_∞ and K conditional on l and t could be formed for a group of animals, each with its own pair of von Bertalanffy parameters ($L_{\infty i}$, K_i) retained throughout its life, or for animals of a given length or age (Sainsbury 1980). It is further assumed that this conditional distribution is identical over the length or age range considered. The length increment I_i of the ith animal of initial length l for time at liberty t is

$$I_i = (L_{\infty i} - l)(1 - e^{-K_i t})$$

and for a group of animals randomly selected from the population, the expected value of length increment is

$$(3)\quad E[I|l,t] = E[(L_\infty - l)(1 - e^{-Kt})]$$

with variance

$$(4)\quad V[I|l,t] = V[(L_\infty - l)(1 - e^{-Kt})].$$

If L_∞ and K have the bivariate normal distribution with means $\bar{L}_\infty$ and $\bar{K}$, variances $\sigma^2_{L_\infty}$ and σ^2_K, and correlation coefficient ρ, equations 3 and 4 become, respectively:

$$(5)\quad E[I|l,t] = E[(L_\infty - l)(1 - e^{-Kt})]$$

$$= \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} \frac{1}{2\pi\sigma_{L_\infty}\sigma_K\sqrt{1-\rho^2}}(L_\infty - l)(1 - e^{-Kt}) e^{-\frac{1}{2(1-\rho^2)}\left[\frac{(L_\infty-\bar{L}_\infty)^2}{\sigma^2_{L_\infty}} - 2\rho\frac{(L_\infty-\bar{L}_\infty)(K-\bar{K})}{\sigma_{L_\infty}\sigma_K} + \frac{(K-\bar{K})^2}{\sigma^2_K}\right]} dL_\infty\, dK$$

$$= \bar{L}_\infty - l - (\bar{L}_\infty - l - \sigma_{L_\infty}\sigma_K\rho t) e^{-\bar{K}t + \frac{1}{2}(\sigma_K t)^2}$$

$$(6)\quad V[I|l,t] = V[(L_\infty - l)(1 - e^{-Kt})]$$

$$= E\{[(L_\infty - l)(1 - e^{-Kt})]^2\} - \{E[(L_\infty - l)(1 - e^{-Kt})]\}^2$$

$$= \sigma^2_{L_\infty} + 2[\sigma_{L_\infty}\sigma_K\rho t(\bar{L}_\infty - l - \sigma_{L_\infty}\sigma_K\rho t) - \sigma^2_{L_\infty}] e^{-\bar{K}t + \frac{1}{2}(\sigma_K t)^2} + [(\bar{L}_\infty - l - 2\sigma_{L_\infty}\sigma_K\rho t)^2 + \sigma^2_{L_\infty}] e^{-2\bar{K}t + 2(\sigma_K t)^2}$$
$$- (\bar{L}_\infty - l - \sigma_{L_\infty}\sigma_K\rho t)^2 e^{-2\bar{K}t + (\sigma_K t)^2}.$$

Model Behaviour

Certain aspects of predictive differences between equations 2 and 5 may be examined by making assumptions about their difference, D, with

$$(7)\quad D = -\bar{L}_\infty + (\bar{L}_\infty - l - \sigma_{L_\infty}\sigma_K\rho t) e^{-\bar{K}t + \frac{1}{2}(\sigma_K t)^2} + L_\infty - (L_\infty - l)e^{-Kt}.$$

Since K and L_∞ are often interpreted as means or averages, one may assume that $K = \bar{K}$ and $L_\infty = \bar{L}_\infty$. Under this assumption, equation 7 becomes

$$(8)\quad D = \left[(\bar{L}_\infty - l - \sigma_{L_\infty}\sigma_K\rho t) e^{\frac{1}{2}(\sigma_K t)^2} - (\bar{L}_\infty - l)\right] e^{-\bar{K}t}$$

and $D > 0$, $D = 0$, and $D < 0$ correspond respectively to overestimation, equivalence, and underestimation by equation 2 if equations 5 and 6 are correct, or vice versa. This predictive difference can also be considered relative. I will assume below that equations 5 and 6 are "correct". The argument is reversed if the opposite is true or rephrased otherwise. Biological and mathematical contexts require nonnegativity of all variables and parameters in equation 8 except for $-1 \le \rho \le 1$. Since $-1 \le \rho \le 0$ (Pauly 1987; Burr 1988), D is usually nonnegative, and use of equations 1 and 2 usually overestimates mean length at age and length increment for initial length and time at liberty respectively (Table 1). Since $T - T_0 > 0$ for any animal, and $t > 0$ for any tagged, released, and subsequently recaptured animal, and because of individual variability of K in natural animal populations (i.e., $\sigma^2_K > 0$), I have excluded in Table 1 trivial cases of $D = 0$ resulting from $\sigma^2_K = 0$, $T - T_0 = 0$, or $t = 0$.

A few other predictions are evident. D linearly decreases with ρ and increases with $\bar{L}_\infty$. Thus, overestimation is more serious intraspecifically in individuals or interspecifically in species of large sizes than of small sizes; the opposite applies to underestimation. Similarly, D linearly increases if $-1 \le \rho < 0$ or decreases $0 < \rho \le 1$ with σ_{L_∞}. However, if K does not vary among individuals (i.e., $\sigma^2_K = 0$ and $\rho = 0$), neither $\bar{L}_\infty$ nor σ_{L_∞} affects D. D decreases linearly with l: overestimation, if present, is more serious for animals of small initial length, and underestimation is more serious for animals of large initial length. As $\bar{K}$ increases, D exponentially decreases if $u_0 > 0$ or increases if $u_0 < 0$ from u_0 at $\bar{K} = 0$

TABLE 1. Predictive differences between equations 2 and 5 in terms of certain parameters and variables.

Parameter	Threshold	Overestimation	Equivalence	Underestimation	Remarks
ρ	$[(\bar{L}_\infty - l - \sigma_{L_\infty}\sigma_K t)e^{\frac{1}{2}\sigma_K^2 t^2} - (\bar{L}_\infty - l)]e^{-\bar{K}t} > 0$	Linearly decreased only			
	$[(\bar{L}_\infty - l - \sigma_{L_\infty}\sigma_K t)e^{\frac{1}{2}\sigma_K^2 t^2} - (\bar{L}_\infty - l)]e^{-\bar{K}t} = 0$	First linearly decreased	Then equivalent at $\rho = 1$		
	$[(\bar{L}_\infty - l - \sigma_{L_\infty}\sigma_K t)e^{\frac{1}{2}\sigma_K^2 t^2} - (\bar{L}_\infty - l)]e^{-\bar{K}t} < 0$	First linearly decreased at $-1 \le \rho < \rho_0$	Then equivalent at $\rho = \rho_0$	Finally linearly increased at $\rho_0 < \rho \le 1$	$\rho_0 = \dfrac{\bar{L}_\infty - l}{\sigma_{L_\infty}\sigma_K t}\left(1 - e^{-\frac{1}{2}\sigma_K^2 t^2}\right)$
$\bar{L}_\infty$	$[-(l + \sigma_{L_\infty}\sigma_K \rho t)e^{\frac{1}{2}\sigma_K^2 t^2} + l]e^{-\bar{K}t} > 0$	Linearly increased only			
	$[-(l + \sigma_{L_\infty}\sigma_K \rho t)e^{\frac{1}{2}\sigma_K^2 t^2} + l]e^{-\bar{K}t} = 0$	Then linearly increased at $\bar{L}_\infty > 0$	First equivalent at $\bar{L}_\infty = 0$		
	$[-(l + \sigma_{L_\infty}\sigma_K \rho t)e^{\frac{1}{2}\sigma_K^2 t^2} + l]e^{-\bar{K}t} < 0$	Finally linearly increased at $\bar{L}_\infty > \bar{L}_{\infty 0}$	Then equivalent at $\bar{L}_\infty = \bar{L}_{\infty 0}$	First linearly decreased at $\bar{L}_\infty < \bar{L}_{\infty 0}$	$\bar{L}_{\infty 0} = l + \dfrac{\sigma_{L_\infty}\sigma_K \rho t}{1 - e^{-\frac{1}{2}\sigma_K^2 t^2}}$
σ_{L_∞}	$-1 \le \rho < 0$	Linearly increased only			
	$0 < \rho \le 1$	First linearly decreased at $\sigma_{L_\infty} < \sigma_0$	Then equivalent at $\sigma_{L_\infty} = \sigma_0$	Finally linearly increased at $\sigma_{L_\infty} > \sigma_0$	$\sigma_0 = \dfrac{\bar{L}_\infty - l}{\sigma_K \rho t}\left(1 - e^{-\frac{1}{2}\sigma_K^2 t^2}\right)$
$\bar{K}$		Exponentially decreased at $u_0 > 0$	Equivalent at $u_0 = 0$	Exponentially decreased at $u_0 < 0$	$u_0 = (\bar{L}_\infty - l - \sigma_{L_\infty}\sigma_K \rho t)e^{\frac{1}{2}\sigma_K^2 t^2} - (\bar{L}_\infty - l)$
l	$[(\bar{L}_\infty - \sigma_{L_\infty}\sigma_K \rho t)e^{\frac{1}{2}\sigma_K^2 t^2} - \bar{L}_\infty]e^{-\bar{K}t} < 0$			Only linearly increased at $l \ge 0$	
	$[(\bar{L}_\infty - \sigma_{L_\infty}\sigma_K \rho t)e^{\frac{1}{2}\sigma_K^2 t^2} - \bar{L}_\infty]e^{-\bar{K}t} = 0$		Equivalent at $l = 0$	Linearly increased at $l > 0$	
	$[(\bar{L}_\infty - \sigma_{L_\infty}\sigma_K \rho t)e^{\frac{1}{2}\sigma_K^2 t^2} - \bar{L}_\infty]e^{-\bar{K}t} > 0$	First linearly decreased at $0 < l < l_0$	Then equivalent at $l = l_0$	Finally linearly increased at $l > l_0$	$l_0 = \bar{L}_\infty - \dfrac{\sigma_{L_\infty}\sigma_K \rho t}{1 - e^{-\frac{1}{2}\sigma_K^2 t^2}}$

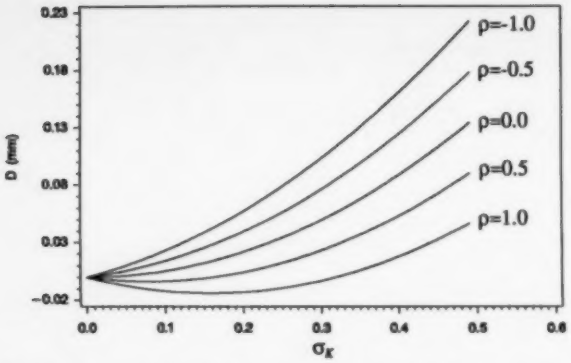

FIG. 1. An example of D varying with σ_K at different ρs for male *M. endeavouri*, with $\bar{L}_\infty = 32.03$ mm, $\sigma^2_{L_\infty} = 1.67$ mm^2, $\bar{K} = 2.38 \cdot \text{yr}^{-1}$, $t = 0.25$ yr, and $l = 0.00$ mm.

towards zero as $\bar{K} \to \infty$ with $u_0 = (\bar{L}_\infty - l - \sigma_{L_\infty}\sigma_K \rho t)e^{\frac{1}{2}\sigma^2_K t^2} - (\bar{L}_\infty - l)$, suggesting that long-lived animals such as elephants and whales (with small $\bar{K}$) are more affected by either overestimation or underestimation than short-lived animals such as rotifers, cladocerans, and penaeids (with large $\bar{K}$).

For σ_K, D is minimized at

$$\sigma_{\min} = \frac{1}{2\sigma_{L_\infty}\rho t}\left(\bar{L}_\infty - l - \sqrt{(\bar{L}_\infty - l)^2 - 4\sigma^2_{L_\infty}\rho^2}\right)$$

and maximized at

$$\sigma_{\max} = \frac{1}{2\sigma_{L_\infty}\rho t}\left(\bar{L}_\infty - l + \sqrt{(\bar{L}_\infty - l)^2 - 4\sigma^2_{L_\infty}\rho^2}\right).$$

Here again, ρ acts as a switch variable. If $-1 \le \rho < 0$, both extrema fall on the left half of the σ_K–D plane (i.e., all $\sigma_K \le 0$), with $\sigma_{\max} < \sigma_{\min}$. Because $\sigma_K \ge 0$ for all animal populations, in this case, D can only progressively increase from zero at $\sigma_K = 0$, leading first to equivalence at $\sigma_K = 0$ and then to a progressively increased overestimation. If $0 < \rho \le 1$, both extrema fall on the right half of the σ_K–D plane (i.e., all $\sigma_K \ge 0$), with $\sigma_{\min} < \sigma_{\max}$. In this case, D first decreases with σ_K from zero at $\sigma_K = 0$ to a minimum at $\sigma_K = \sigma_{\min}$, then increases through $D = 0$ at some σ_K to a maximum at $\sigma_K = \sigma_{\max}$, and then decreases sharply through $D = 0$ at some σ_K and finally to $D < 0$. Thus, there is a progressively increased, then maximized, and finally reduced underestimation with increasing σ_K until equivalence occurs at some σ_K, followed by a progressively increased overestimation until $\sigma_K = \sigma_{\max}$, where it drops sharply and is reduced to zero at some σ_K before changing into a sharply increased underestimation. However, animals are normally unlikely to experience a σ_K well below $\sigma_{\max}$. An example of D varying with σ_K at different ρs is depicted in Fig. 1 for male *M. endeavouri*, with $\bar{L}_\infty = 32.03$ mm, $\sigma^2_{L_\infty} = 1.67$ mm^2, $\bar{K} = 2.38 \cdot \text{yr}^{-1}$, $t = 0.25$ yr, and $l = 0.00$ mm.

Finally, T or t is related to D by transcendental equations that can only be solved numerically except for such trivial cases as $\sigma_K = 0$. For male *M. endeavouri*, with $\bar{L}_\infty = 32.03$ mm, $\sigma^2_{L_\infty} = 1.67$ mm^2, $\rho = -0.44$, $\bar{K} = 2.38 \cdot \text{yr}^{-1}$, and $\sigma^2_K = 0.96 \cdot \text{yr}^{-2}$, D first increases with T to a maximum at some T, then decreases to some minimum at some other T, and finally increases over an age below 3 yr. In this example, $D > 0$ or overestimation occurs throughout the age range.

Another assumption about D, and its parameters and variables, is that equations 2 and 5 describe the same mean length at age, or mean length increment, i.e., $D = 0$. This formulation may also be seen as one set of models being correct, while the other leads to biases in estimates of growth parameters, or simply as their relative biases. Three possibilities arise from equation 7, rewritten as

$$\bar{K} - K = \bar{K} + \frac{1}{t}\ln\left[\frac{L_\infty - \bar{L}_\infty + (\bar{L}_\infty - l - \sigma_{L_\infty}\sigma_K \rho t)e^{-\bar{K}t + \frac{1}{2}(\sigma_K t)^2}}{L_\infty - l}\right],$$

regarding estimates of each pair of growth parameter: $\bar{K} > K$, $\bar{K} < K$, and $\bar{K} = K$ for K and $\bar{K}$, and $\bar{L}_\infty > L_\infty$, $\bar{L}_\infty < L_\infty$, and $\bar{L}_\infty = L_\infty$ for $\bar{L}_\infty$ and L_∞. $\bar{K}$ and $\bar{L}_\infty$ can overestimate, underestimate, or equate K and L_∞, respectively, depending on values of other parameters and variables. If $\bar{L}_\infty = L_\infty$, $\bar{K} - K \ge 0$ at least for $\rho \le 0$, and use of equations 1 and 2 almost always underestimates K, as also noted by Sainsbury (1980). If $\bar{K} = K$, $\bar{L}_\infty > L_\infty$ at least for $\rho \le 0$, and use of equations 1 and 2 almost always underestimates L_∞.

Generally, $V[l|l,t]$ changes quadratically with ρ, $\bar{L}_\infty$, l, and σ_{L_∞}, with a minimum at

$$\rho = \frac{(\bar{L}_\infty - l)(1 - 2uv^3 + uv)}{\sigma_{L_\infty}\sigma_K t(2 - 4uv^3 + uv)},$$

$$\bar{L}_\infty = l + \sigma_{L_\infty}\sigma_K \rho t\left[2 + \frac{uv - 1}{uv(v^2 - 1)}\right],$$

$$l = \bar{L}_\infty - \sigma_{L_\infty}\sigma_K \rho t\left[2 + \frac{uv - 1}{uv(v^2 - 1)}\right],$$

and

$$\sigma_{L_\infty} = \frac{\sigma_K \rho t(\bar{L}_\infty - l)uv(-1 + 2uv^3 - uv)}{1 - 2[1 + (\sigma_K \rho t)^2]uv + [1 + 4(\sigma_K \rho t)^2]u^2v^4 - (\sigma_K \rho t)^2 u^2 v^2},$$

respectively, with $u = e^{-\bar{K}t}$ and $v = e^{(\sigma_K t)^2/2}$. However, it is independent of l if $\sigma^2_K = 0$ and decreases with l essentially in proportion to, if $\rho = 0$, or quadratically with, if $\rho \ne 0$, $(\bar{L}_\infty - l)^2$ for a constant time at liberty if $\sigma^2_K > 0$. These variations could be used to gain a general idea of the level of variability of K. For example, a general increase in variance of

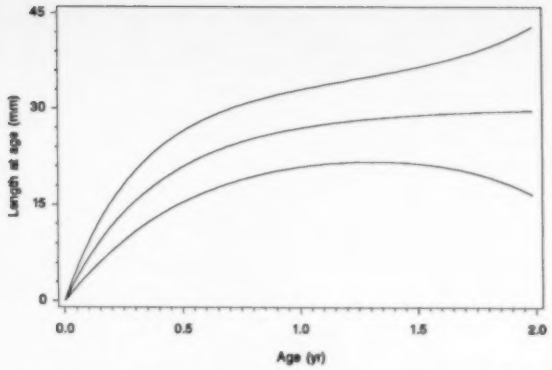

FIG. 2. Mean length at age and its variance (equations 5 and 6) in *M. endeavouri*, with $\bar{L}_\infty = 32.03$ mm, $\sigma^2_{L_\infty} = 1.67$ mm^2, $\rho = -0.44$, $\bar{K} = 2.38 \cdot yr^{-1}$, $\sigma^2_K = 0.96 \cdot yr^{-2}$, and $T_0 = 0$ yr, with $E[l_T|T] \pm \sqrt{V[l_T|T]}$ as upper and lower bounds.

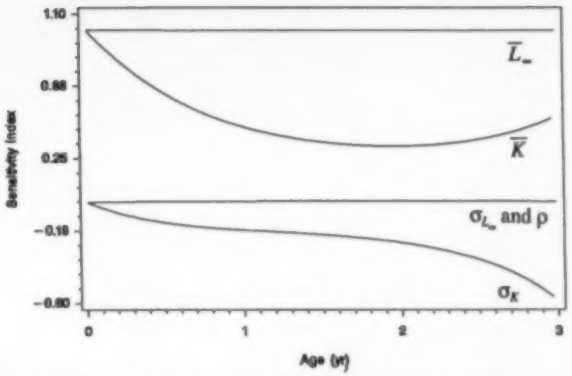

FIG. 3. Effects of age on normalized sensitivity index for various von Bertalanffy growth parameters in *M. endeavouri*, with $\bar{L}_\infty = 32.03$ mm, $\sigma^2_{L_\infty} = 1.67$ mm^2, $\rho = -0.44$, $\bar{K} = 2.38 \cdot yr^{-1}$, and $\sigma^2_K = 0.96 \cdot yr^{-2}$.

length at age with increasing age would suggest a small variance of K. $\bar{K}$ affects $V[l|l,t]$ exponentially, with a minimum or maximum at

$$\bar{K} = \frac{1}{2}\sigma^2_K t - \frac{1}{t}\ln\left\{\frac{\sigma^2_{L_\infty} - \sigma_{L_\infty}\sigma_K\rho t(\bar{L}_\infty - l - \sigma_{L_\infty}\sigma_K\rho t)}{[(\bar{L}_\infty - l - 2\sigma_{L_\infty}\sigma_K\rho t)^2 + \sigma^2_{L_\infty}e^{(\sigma_K t)^2} - (\bar{L}_\infty - l - \sigma_{L_\infty}\sigma_K\rho t)^2}\right\}.$$

Finally, $V[l|l,t]$ varies in a complex manner with σ_K, and T or t; multiple extrema may exist that can generally be found only numerically. For male *M. endeavouri*, with $\bar{L}_\infty = 32.03$ mm, $\sigma^2_{L_\infty} = 1.67$ mm^2, $\rho = -0.44$, $\bar{K} = 2.38 \cdot yr^{-1}$, and $\sigma^2_K = 0.96 \cdot yr^{-2}$, it increases initially slowly and then abruptly with age (Fig. 2).

Data, Parameter Estimation, and Sensitivity Analysis

Equations 5 and 6 were fitted into Buckworth's (1992) length increment data on the penaeid *M. endeavouri*. The data set was based on 13 308 penaeids of both sexes and of a carapace length range of 19–43 mm tagged with streamer tags and released in the Gulf of Carpentaria, northern Australia, between January and March and between June and July 1984. Of the males tagged, 150 with a carapace length range of 21–34 mm (mean = 28 mm, SD = 3 mm) and with a time at liberty of up to 115 d were recaptured. In this analysis, only 74 with a time at liberty of 0–101 d (mean = 47 d, SD = 24 d), a carapace length range of 22–32 mm (mean = 27 mm, SD = 3 mm), and a carapace length increment range of 1–6 mm (mean = 2 mm, SD = 1 mm) were used because males apparently grow more smoothly than females and because of incomplete or unpredictable information for others. The data set was not the best choice although VBGEs have frequently been used to model penaeid growth data because crustaceans grow incrementally. However, VBGEs might reflect an average growth trend in these animals. Further, the small size of penaeids means that estimates of L_∞ and $\bar{L}$ are unlikely to be too biased.

$\bar{L}$, σ_{L_∞}, $\bar{K}$, σ_K, ρ, and a model error term (or residual) σ^2_e were estimated by maximizing the likelihood function

$$L = \prod_{i=1}^{n}[2\pi V[l|l,t]_i + \sigma^2_e]^{-\frac{1}{2}} e^{-\frac{[l_i - E[l|l,t]_i]^2}{2[V[l|l,t]_i + \sigma^2_e]}}$$

at $\bar{L}_\infty = 32.03$ mm, $\sigma^2_{L_\infty} = 1.67$ mm^2, $\rho = -0.44$, $\bar{K} = 2.38 \cdot yr^{-1}$, $\sigma^2_K = 0.96 \cdot yr^{-2}$, and $\sigma^2_e = 0.64$ mm^2. By contrast, fitting of conventional von Bertalanffy model of Fabens form resulted in $L_\infty = 34.03$ mm and $K = 2.02 \cdot yr^{-1}$, with a ρ of -0.94 being given in the correlation matrix. Analytic sensitivity analysis (Tomović 1963) of equation 5 for length at age (let $t = T - T_0$, $T_0 = 0$, and $l = 0$) was conducted based on above estimates by calculating normalized sensitivity index

$$\frac{p_i}{E[l|l,t]}\frac{\partial E[l|l,t]}{\partial p_i}$$

for individual parameters p_i given their first partial derivatives $\partial E[l|l,t]/\partial p_i$. The index ranged from 1.00492 to 1.01673 for $\bar{L}_\infty$, -0.016726 to -0.0049210 for σ_{L_∞}, -0.016726 to -0.0049210 for ρ, 0.31919 to 1.00713 for $\bar{K}$, and -0.62137 to -0.020785 for σ_K for an age range of 0–3 yr (Fig. 3). Equation 5 is then sensitive to changes in $\bar{L}_\infty$, $\bar{K}$, and σ_K and relatively insensitive to those in σ_{L_∞} and ρ.

Discussion

This work presents VBGEs for length at age and length increment for initial length and time at liberty derived by assuming L_∞ and K to follow the joint bivariate normal distribution with a correlation coefficient. Under very general conditions, the sum (or average) of a number of random variables is approximately normally distributed, and such approximation can be quite good even if that number is relatively small. The above assumption would be at least

approximately valid because both K and L_∞ can be regarded as the sum (or average) of numerous (e.g., genetic, phenotypic, and behavioural) random components. The applicability of the models derived from it for biological populations can be assessed by the age or time at liberty at which biologically unrealistic negative growth starts, T_n, and that at which the growth models curve downwards (animals normally grow nonnegatively for rigid growth variables like body length):

$$T_c = \frac{1}{2\sigma_{L_\infty}\sigma_K^2\rho}\left\{\sigma_K(\bar{L}_\infty - l) + \sigma_{L_\infty}\rho\bar{K} - \sqrt{[\sigma_K(\bar{L}_\infty - l) - \sigma_{L_\infty}\rho\bar{K}]^2 - (2\sigma_{L_\infty}\sigma_K\rho)^2}\right\} \approx \frac{\bar{K}}{\sigma_K^2}.$$

T_n cannot generally be solved exactly from equation 5, but an approximate formula

$$T_n \approx \frac{2\bar{K}}{\sigma_K^2} \approx 2T_c$$

would suffice. Thus, σ_K^2 must be sufficiently small with respect to $\bar{K}$, such that T_c is greater than or equal to the life span of an animal, to avoid biologically unrealistic mean lengths and mean length increments. For *M. endeavouri*, $T_n \approx 4.96$ yr and $T_c \approx 2.48$ yr, while its life span and those of most penaeids are only 12 mo or so! For those species at least, the bivariate normality assumption has given a viable set of growth models, which may be seen as an extension of, and alternative to, Sainsbury VBGEs. It follows that Sainsbury's (1980) concerns about negative mean lengths and mean length increments were justified, but were nonetheless unnecessary at least for those species.

Concerns about negative correlation between L_∞ and K were rational, but again unnecessary in terms of predictions of mean length and length increment. Although they might be strongly negatively correlated in other species, the analysis of model behaviour and sensitivity reveals that ρ is of minor importance in model predictions for *M. endeavouri*. Furthermore, the relatively small correlation coefficient, again at least for *M. endeavouri*, indicates that the correlation might be weaker than previously thought. The relatively low correlation and little variability in L_∞ indicate that a special case of equations 5 and 6 for $\sigma_{L_\infty}^2 = 0$ and $\rho = 0$ is adequate for describing growth of this and probably other species of penaeids.

However, an important message comes from this study. Correlation between L_∞ and K of a VBGE that varies among individuals of a population (where expectation is taken over all members of the population) is not the same as, and must be distinguished from, that between estimates of both parameters in the relationship for a single individual or individuals of a population with an identical pair of von Bertalanffy parameters. The phenomenon is not unique to VBGEs, but also exists, say, in the simple linear regression. Thus, negative correlations generated routinely by some computer packages in fitting conventional VBGEs generally refer to the correlation between the parameter estimates and not to that between individual values (of L_∞ and K) that exist within an animal population, and must be interpreted cautiously.

Another major problem to emerge from examining individual variability in growth parameters is changes in conditional (on age or initial length) distributions of K and L_∞. That such conditional distributions do not alter is a key assumption in deriving the equations provided, and any violation will result in departures from them, and also from the traditional forms of the equations. For length at age data, sampling bias and differential mortality may affect age specific distributions of both parameters (Ricker 1975). For length increment data, age composition of the sampled group, and truncated distribution of L_∞ when large animals are considered may also be involved (Sainsbury 1980). Lastly, one must keep in mind that variability in growth parameters is not the only factor affecting the predictability of growth models, but is compounded with that from other sources, such as ageing errors (Sainsbury 1980), tagging effects (Xiao 1994), and biased data. Thus the growth models would be more useful if variability from all other sources were controlled.

Acknowledgments

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Improvements to the Diurnal Upstream–Downstream Dissolved Oxygen Change Technique for Determining Whole-Stream Metabolism in Small Streams

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Marzolf, E.R., P.J. Mulholland, and A.D. Steinman. 1994. Improvements to the diurnal upstream–downstream dissolved oxygen change technique for determining whole-stream metabolism in small streams. Can. J. Fish. Aquat. Sci. 51: 1591–1599.

Whole-stream metabolism in a first-order stream was measured using upstream–downstream changes in dissolved oxygen (DO) concentration measured at 1-min intervals over a 40-h period. The measured change in DO was corrected for reaeration flux using a reaeration coefficient determined from injections of conservative and volatile tracers. The whole-stream metabolism measurement was compared in the spring with in situ chamber measurements performed a few days later in the same stream reach. Chamber measurements of community respiration extrapolated to a 24-h period (CR_{24}) were about one third the whole-stream measurements, while gross primary production (GPP) measured at midday in the chambers was roughly 20% less than the whole-stream estimate. Whole-stream GPP was higher during the spring just prior to forest canopy closure than in summer or autumn. Community respiration exceeded whole-stream GPP on all dates and was greatest during the summer. Our results suggest that this whole-stream approach provides a measure of total stream metabolism that is relevant to other stream ecosystem processes measured on reach scales, such as nutrient spiralling.

Le métabolisme global du cours d'eau dans un cours d'eau de premier ordre a été mesuré en utilisant les changements amont–aval de la concentration d'oxygène dissous (OD) mesurés à des intervalles de 1 min pendant une période de 40 h. Le changement mesuré de la concentration d'OD a été corrigé pour tenir compte du flux de réaération à l'aide d'un coefficient de réaération déterminé après l'injection de traceurs stables et volatils. La mesure du métabolisme à l'échelle du cours d'eau a été comparée au printemps avec les mesures effectuées in situ dans des chambres quelques jours plus tard dans le même tronçon du cours d'eau. Les mesures en chambre de la respiration globale, extrapolées à une période de 24 h (CR_{24}) sont égales environ au tiers de mesures à l'échelle du cours d'eau, tandis que la production primaire brute (PPB) mesurée au milieu de la journée dans les chambres est inférieure d'environ 20 % à l'estimation pour l'ensemble du cours d'eau. La PPB pour l'ensemble du cours d'eau est plus élevée au printemps, juste avant la fermeture du couvert forestier, qu'en été ou en automne. La respiration globale est supérieure à la PPD à l'échelle du cours d'eau en toute saison et atteint son maximum en été. Nos résultats semblent indiquer que cette approche du cours d'eau à l'échelle globale permet une mesure du métabolisme total du cours d'eau qui présente un intérêt pour d'autres processus écosystémiques du cours d'eau mesurés à l'échelle du tronçon, par exemple le flux en hélice des substances nutritives.

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There are two commonly used approaches in measuring the metabolism in a stream: (1) chamber studies in which a portion of a stream community is enclosed and changes in dissolved oxygen (DO) concentration, pH, or the uptake of a radioisotope are measured and (2) in situ measurements of changes in DO or pH in open channels over a diurnal period at either one or two sites. The methods may not necessarily give the same results and the method chosen will influence how data are interpreted due to the trade-offs inherent in each.

Chamber measurements of benthic metabolism rely on confining and recirculating water around a subsample of the benthos (Hall and Moll 1975; Lyford and Gregory 1975; Bott et al. 1978; Lamberti et al. 1989). These methods have several advantages which often make their use attractive, including the ability to replicate treatments, to ignore reaeration concerns (especially if using radioisotope uptake methods), and to measure metabolism rates of particular taxa or assemblages.

However, chamber measurements also suffer from several potentially significant problems due to artifacts of enclosure. Such problems include nonrepresentative sampling of the stream, disruption of the enclosed benthic community, creation of unnatural environmental conditions (e.g., water velocity, turbulence, temperature, light), the generation of closed-system artifacts such as nutrient depletion, and problems with scaling up the results to the whole system (Hall and Moll 1975; Bott et al. 1978). Differences between chamber and ambient conditions in light, water velocity, DO, or nutrients may affect metabolism (Horner and Welch 1981;

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Horner et al. 1983, 1990; Bothwell 1985, 1988, 1989; Hickey 1988).

In ecos (sensu Odom) methods based on diurnal changes in DO concentration were suggested decades ago by Odum (1956) and Odum and Hoskin (1958). As with the chamber measurements, open-system, whole-stream methods have advantages and disadvantages. On the positive side, they do not require the removal or disturbance of the benthos and include all components of the benthic, water column, and hyporheic communities. In addition, environmental conditions are unaffected by the measurement itself. Such conditions allow direct comparison with other reach-scale measurements such as nutrient spiralling or secondary production.

However, there are several disadvantages to the whole-stream method. First, although it is possible to do repeated measurements, there can be no true replication. Second, this method can be applied only where there is little lateral inflow so that only instream processes are responsible for changes in DO. Thus, if comparisons are to be made between days or seasons, measurements need to be made during similar weather conditions. Third, corrections for air–water exchange of oxygen (reaeration) are uncertain, particularly in reaches with high surface turbulence of where high algal biomass results in photosynthetic oxygen bubble formation. Fourth, if only microbial metabolism is of interest, some sort of correction for macrofauna and macrophyte respiration and macrophyte photosynthesis will be necessary.

Probably the greatest difficulties with in ecos methods have been inaccurate corrections for the reaeration flux of oxygen and problems associated with precisely measuring small DO changes. Many reaeration estimates have relied on either predictive equations based on diurnal oxygen curves (Odum and Hoskin 1958) or hydraulic characteristics (Negulescu and Rojanski 1969; Bennett and Rathbun 1972; Stedfast and Draper 1986). However, these equations can result in inaccurate estimates for the reaeration coefficients (RC) of low-order (Stedfast and Draper 1986; Genereux and Hemond 1992), oligotrophic (Hall and Moll 1975), or high-gradient streams (Genereux and Hemond 1990; Wanninkhof et al. 1990). Various empirical approaches to estimate RC have been tried, including using N_2-filled floating domes (Copeland and Duffer 1964; Duffer and Dorris 1966; Hall 1972) and various volatile tracers injected into the stream reach (Rathbun et al. 1978). The use of floating domes in streams is problematic due to surface heterogeneity and turbulence. Early volatile tracer studies used radioactive compounds but their use has not been widespread for obvious environmental reasons.

Wanninkhof et al. (1990) and Genereux and Hemond (1992) have demonstrated the value of a direct approach using injections of volatile tracers (SF_6 and propane, respectively) and conservative tracers (for travel time and lateral inflow estimates) to measure the RC in small streams. The volatile tracer approach avoids the limitations of dome incubation methods and the problems associated with predictive equations and radioisotopes.

Direct RC measurements, combined with improvements in the precision of instruments for measuring DO concentration, have the potential for great improvement in the accuracy of in ecos, whole-stream metabolism measurements for discrete stream reaches. This study was undertaken to examine stream metabolism measured by an upstream–downstream diurnal DO method that utilizes direct measurement of the reach RC in a low-order woodland stream. Whole-stream metabolism measurements were then compared with measurements made using in situ incubation chambers and related to biotic and abiotic factors influencing metabolism.

Methods

Study Site

Measurements were made on a 62-m reach in the first-order portion of Walker Branch, a forest stream in eastern Tennessee (Fig. 1). This reach consists of alternating runs and pools and has a total wetted area of approximately 136 m^2 and an average depth of approximately 5 cm at baseflows varying from 3 to 10 $L \cdot s^{-1}$. The substrata consist of gravel, cobble, and bedrock. Due to intense herbivory by the snail *Elimia clavaeformis*, algal biomass is low and dominated by grazer-resistant forms such as basal cells of *Stigeoclonium* sp. (Steinman 1992; Rosemond 1993; Rosemond et al. 1993). Bryophytes are distributed patchily within the stream, and Steinman and Boston (1993) estimated that they cover 10–20% of the substrate. Springs located about 25 m above the reach generate a significant portion of the baseflow within the reach (Wanninkhof et al. 1990; Genereux et al. 1993). Spring water is undersaturated with respect to oxygen and has little annual variation in temperature. For a more detailed description of Walker Branch and its watershed, see Johnson and Van Hook (1989).

General Approach

Stream metabolism was measured using DO measurements at the upstream and downstream ends of the reach at 1-min intervals over a period of approximately 40 h (Odum 1956). Changes in DO between upstream and downstream were considered to be the result of two processes, metabolism (photosynthesis and respiration) and the reaeration flux of oxygen. Lateral inflow was shown to contribute <9% to the flow in this reach based on the conservative tracer injections. Chemical oxygen demand was considered to be insignificant. Neither was included in the calculations. The metabolic rate was calculated as the difference between the upstream and downstream DO after correcting for the reaeration flux within the reach during the same time interval.

The reaeration flux depends on the RC and the DO deficit ($DO_{deficit}$) of the stream water. The RC, k_{oxygen} (d^{-1}), was measured by comparing the change in concentration of a volatile tracer relative with that of a conservative tracer within the reach following the method of Genereux and Hemond (1992). This method required the continuous injection of both (although not necessarily at the same time) a volatile tracer (propane) and a conservative tracer (Cl^- added as a NaCl solution) into the stream. Both tracers were introduced a sufficient distance above the upstream site (~10 m) to be uniformly dispersed by the time they reached the upstream site. Samples for volatile tracer concentration were taken only after a steady state was reached along the entire reach. The following equation was used to calculate the propane exchange coefficient, $k_{propane}$:

$$(1) \quad k_{propane}\,(\mathrm{min}^{-1}) = \tau^{-1} \times \ln\left(\frac{G_1 \times C_2}{G_2 \times C_1}\right)$$

where τ = stream water travel time between sites (minutes),

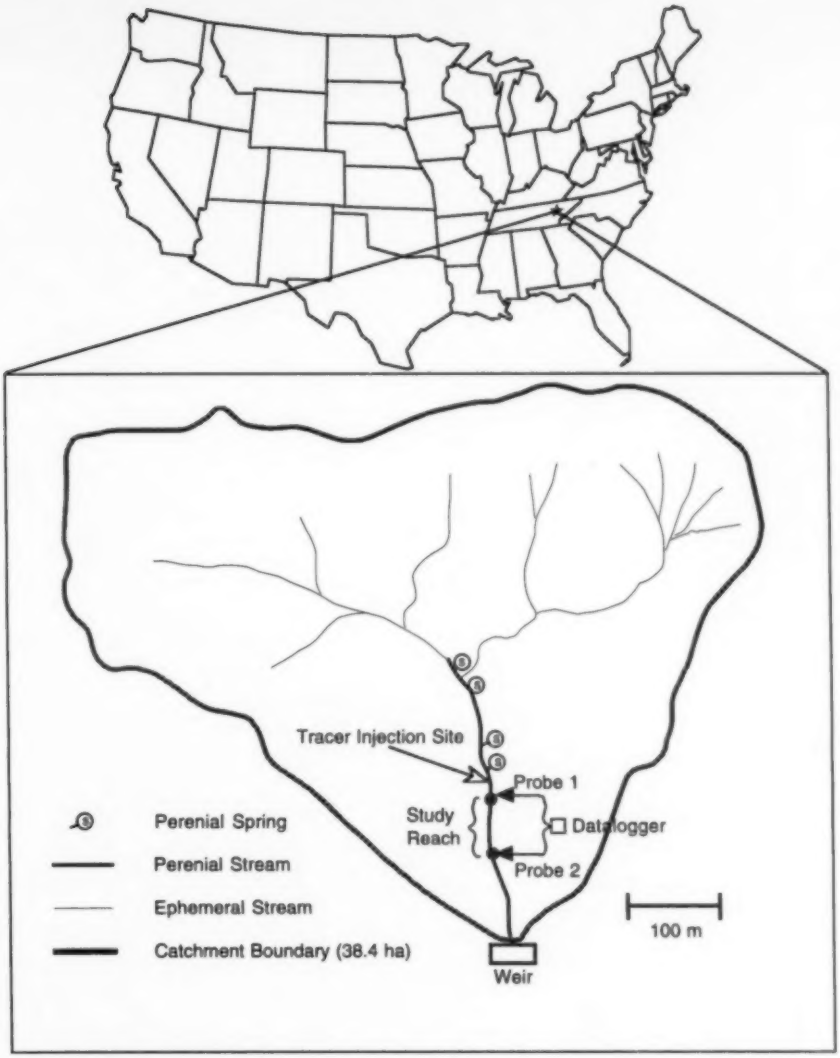

FIG. 1. West fork of the Walker Branch watershed with injection, upstream, and downstream sites of the study reach as well as springs indicated.

G = steady-state concentration of propane at the upstream (1) and downstream (2) sites (this value is in theory corrected for the background concentration, assumed to be zero in Walker Branch and confirmed by previous studies (Genereux et al. 1992)), and C = steady-state conservative tracer concentration at the upstream (1) and downstream (2) sites, corrected for background concentration.

The oxygen exchange coefficient, k_{oxygen}, is proportional to the propane exchange coefficient and is equal to $1.39 \times k_{propane}$ (Rathbun et al. 1978). Rathbun et al. (1978) demonstrated that this relationship is independent of mixing conditions, temperature, and water quality.

The actual instantaneous oxygen flux into or out of the stream reach is calculated using equation 2:

$$(2)\quad \text{Reaeration flux} = DO_{deficit} \times e^{-k_{oxygen} \times \tau}$$

where k_{oxygen} = RC (per minute), $DO_{deficit} = DO_{saturated} - DO_{measured}$ (milligrams of O_2 per litre), and τ = stream water travel time between sites (minutes). The $DO_{deficit}$ is the difference between the calculated oxygen saturation value and the average of the observed upstream and downstream DO measurements. The oxygen saturation value is calculated using the average reach water temperature and the atmospheric pressure at that time.

The RC is assumed to be constant during a particular 40-h period. However, the actual oxygen flux due to reaeration will change in response to changes in water temperature (via changes in the oxygen saturation value) and ambient DO concentrations. The instream flow rate, lateral inflow, and the reach travel time were determined from the conservative tracer data as described in the detailed methodology section below.

Detailed Methodology

A concentrated NaCl solution was injected into the stream to determine instream flow rate, lateral inflow, and the reach water travel time (τ). Conductivity measurements, which are easier to make, may be substituted for actual Cl^- measurements (Stream Solute Workshop 1990) when determining these hydraulic parameters. We introduced a NaCl solution at a constant rate sufficient to increase stream water conductivity by at least 100 μS using either a 50-L Marriotte bottle or a battery-powered peristaltic pump (Cole-Parmer, model 7533-20). Conductivity was measured using Cole-Parmer (C-P models PCM1 and 1481-40) or YSI conductivity meters (model YSI 32).

The reach water travel time was calculated as the time interval required for the conservative tracer pulse to travel between the upstream and downstream sites. The time at each site was calculated as when the conductivity reached one half of its steady-state value (half height). This travel time calculation determined the sampling interval between the upstream and downstream sites for propane and later for calculating the change in DO concentration. Lateral inflow was determined as the percentage decrease between the upstream and downstream sites in steady-state conductivity values (corrected for background values). Streamflow was calculated using either the resultant conservative tracer concentration of the stream water, concentration of the injection solution and the injection rate, or a model developed by Genereux et al. (1993) that relates streamflow at the midpoint of the study reach to that at a weir 115 m below the study reach. Accurate estimates of flow are necessary because flow is used to scale the measured oxygen changes to values per unit of time. DO, temperature, and RC measurements were made within 3 d of each other during stable flow periods.

Propane was bubbled into the stream from a 20-lb tank of HD-5 (the common commercial mixture, initially consisting of 96% propane and 4% ethane) through a weighted air stone. The propane tank regulator pressure was set at 35 kPa and was constant throughout the injection ensuring a constant injection rate (Genereux and Hemond 1992). Because ethane is more volatile than propane, a new tank will deliver a greater proportion of ethane (and therefore a lower proportion of propane). Because it is important that the water samples be taken while the reach is at steady state with respect to propane concentration, the proportions of gas introduced must also be constant. This was accomplished by out-gassing the first pound of gas from a new tank, which removes the portion containing a higher fraction of ethane. After the stream reached steady-state (at least $3 \times \tau$), samples for propane concentration determination were collected at the upstream site and downstream site. Water samples (50 mL) at the downstream site were collected one reach travel time interval (τ) after collection at the upstream site. This was done so that roughly the same parcel of water was sampled at each site. Samples were collected in 50-mL glass syringes (B-D Yale) equipped with three-way valves and stored cold. Care was taken to ensure that there were no bubbles in the syringes. Within 1 h of sample collection, 5 mL of helium was added to each syringe and the syringes placed on a shaking table at low speed (30 rpm) for approximately 3 h to allow the dissolved propane to equilibrate with the helium headspace. Propane in the helium headspace was measured on a Perkin-Elmer 392B gas chromatograph with a 1-mL sample loop and a 6-ft-long, 2-mm inside diameter separation column packed with 80/100 Porapak Q and equipped with a flame ionization detector. The carrier gas was helium at a flow rate of 100 $mL \cdot min^{-1}$. The oven temperature was set at 130°C, while the interface temperature was 150°C. Retention time of the propane peak was approximately 1 min and it was integrated on a Hewlett-Packard 3390A electronic integrator. The linearity and drift of the analyzer were determined using ethane as a standard gas. The integrated area of the propane peak was used directly in the RC calculations, instead of actual concentrations.

Instream DO concentration and temperatures were measured using two Orbisphere oxygen meters (model 2607) equipped with miniature sensing heads (model 2112). At the beginning of each measurement period, the instruments were air calibrated. Next the probes were positioned at the same point in the stream and fine adjustments were made to each meter so that they all read the same DO concentrations. When possible, the instream calibration was checked during or after the experimental run to correct for any instrument drift. DO measurements were made at sites that were laterally constricted and on bedrock so that the sensors were exposed to well-mixed water, representative of the entire stream cross section. Oxygen and temperature measurements were recorded with a Campbell Scientific datalogger (model CR10) at 1-min intervals, and 5-min averages were computed over approximately 40 h during each measurement period.

The time, temperature, and DO data from the datalogger were copied into a computer spreadsheet along with the flow, travel time, and RC data from the tracer injections. A spreadsheet model was used to generate the whole-stream metabolism measurements after identifying the times of dawn and dusk (see below).

Metabolism Calculations

In the spreadsheet, the average of upstream and downstream temperatures was used to calculate $DO_{saturation}$. Elevation was estimated to the nearest foot from detailed topographic maps (2-ft contours) and was used to calculate the atmospheric oxygen partial pressure using equations from Hutchinson (1957). Alternatively, atmospheric oxygen partial pressure was determined from same-day meteorological observations of barometric pressure, after correcting for elevation differences. A third-order equation ($r^2 > 0.9999$, $p < 0.0001$) was fit to a table of partial pressure, temperature, and oxygen saturation values (Orbisphere Laboratories 1991) to calculate $DO_{saturation}$ from the average stream temperature. The $DO_{deficit}$ was calculated as the difference between $DO_{saturation}$ and the average of the upstream and downstream DO measurements. This averaging process assumed that the upper and lower halves of the reach were equal with respect to changes in DO.

Four metabolic estimates were calculated using the difference in the upstream–downstream DO data:

$$\text{Dark metabolism} = \Delta DO_{dark} - \text{reaeration}$$

$$\text{Net metabolism} = \Delta DO_{light} - \text{reaeration}$$

$$\text{Gross community primary productivity (GPP)} = \text{Net metabolism} + \text{respiration}_{light}$$

$$\text{Community respiration } (CR_{24}) = \text{Avg. night}_{resp} \text{ scaled for 24 h.}$$

The change in DO concentration (ΔDO) was calculated as the difference between values at the upstream site and the downstream site at one travel time interval later (or closest

TABLE 1. Comparison of various predictive equations and the dual tracer method to estimate the reaeration coefficient (d^{-1}) for the study reach on three dates. Estimated values were corrected for average ambient water temperature using the equation of Elmore and West (1961).

Estimation method	10 Apr. 1992	29 July 1992	16 Nov. 1992
Bennett and Rathbun 1972	137.4	115.2	76.9
Churchill et al. 1962	1.7	31.2	17.5
Isaacs and Gaudy 1968	22.0	16.0	8.9
Krenkel and Orlob 1963	84.3	76.1	55.8
Langbein and Durum 1967	14.3	10.4	5.8
Negulescu and Rojanski 1969	11.0	8.4	5.0
O'Connor and Dobbins 1958	70.6	4.0	2.9
Padden and Gloyna 1971	12.7	10.3	6.6
Parkhurst and Pomeroy 1972	38.1	34.8	25.9
This study (dual tracer method)	135.5	128.9	67.0

TABLE 2. Hydraulic and metabolic data for the 62-m, first-order reach of Walker Branch.

Parameter	10 Apr. 1992	29 July 1992	16 Nov. 1992
Flow ($L \cdot s^{-1}$)	6.18	3.03	2.63
Travel time (min)	17.1	24.8	39.7
RC (d^{-1})	135.5	128.9	67.0
Lateral inflow (%)	9.5	4.4	8.3
Average water temperature (°C)	13.6	15.6	10.7
GPP			
g $O_2 \cdot reach^{-1} \cdot d^{-1}$	140.97	8.64	9.72
$\mu g\ O_2 \cdot m^{-2} \cdot s^{-1}$	20.17	1.44	1.62
CR_{24}			
g $O_2 \cdot reach^{-1} \cdot d^{-1}$	−235.95	−343.73	−147.47
$\mu g\ O_2 \cdot m^{-2} \cdot s^{-1}$	−19.69	−28.68	−12.30

5-min interval). $Respiration_{light}$ was estimated by determining the average dark metabolism rate during the nights before and after the day in question and fitting a simple linear regression from the previous night's respiration average (set to 06:00 or dawn) and the following night's average (set to 20:00 or sunset). Using the regression data, it was then possible to estimate the light respiration rate for any time during the day. The estimate of CR_{24} was the average of the two nights respiration scaled to 24 h so as to include $respiration_{light}$. Respiration is reported as negative values indicating oxygen consumption.

Chamber Measurements

Measurements of periphyton metabolism were also made using cylindrical ceramic tiles (1.5 × 1.5 cm) that had been in Walker Branch for at least 6 mo. Fifteen tiles from six locations within the study reach, including both thalweg and backwater sites, were placed in sealed Plexiglas chambers (850-mL volume) equipped with recirculating pumps and incubated in situ. They were collected during two days in mid-April. Metabolism was measured as the change in DO (with the Orbisphere meter) under the light conditions (from 11:30 to 13:30) and during a 2-h period immediately following during which the chambers were covered with opaque material. Although the chamber measurements were conducted 3–4 d after the whole-stream measurements, stream baseflow and weather conditions (mostly clear) were similar during both periods.

Results and Discussion

Improvements in the Diurnal Upstream–Downstream Oxygen Change Method

We have made several improvements to the diurnal upstream–downstream oxygen change technique, including measurement of reach water travel time, high-frequency, high-precision DO measurements, and direct measurement of reaeration flux. By directly measuring the reach water travel time using conservative tracer injections, certain measurements can be be made on the same parcel of water as it traverses the reach. In measurements of the volatile tracer, this reduces the likelihood that slight differences in injection rate or gas proportion will cause errors. In the DO measurements, ΔDO (downstream $DO_{t=0}$ − upstream $DO_{t=-\tau}$) is less likely to be influenced by temporal variability in the DO of streamwater at the upstream site.

High-frequency ($1 \cdot min^{-1}$) measurements at upstream and downstream sites using high-precision (±0.02 mg $O_2\ L^{-1}$) DO meters and a datalogger permits the use of a relatively short stream reach (40–60 m), which in turn minimizes the problems of inflow of groundwater of unknown DO concentration. This also results in much finer scale temporal resolution of DO changes in response to the short-term variations in light that are common in forest streams and an accurate integration of metabolism over this temporal variability.

In low-order streams, especially in reaches just below large groundwater inputs of undersaturated water, such as

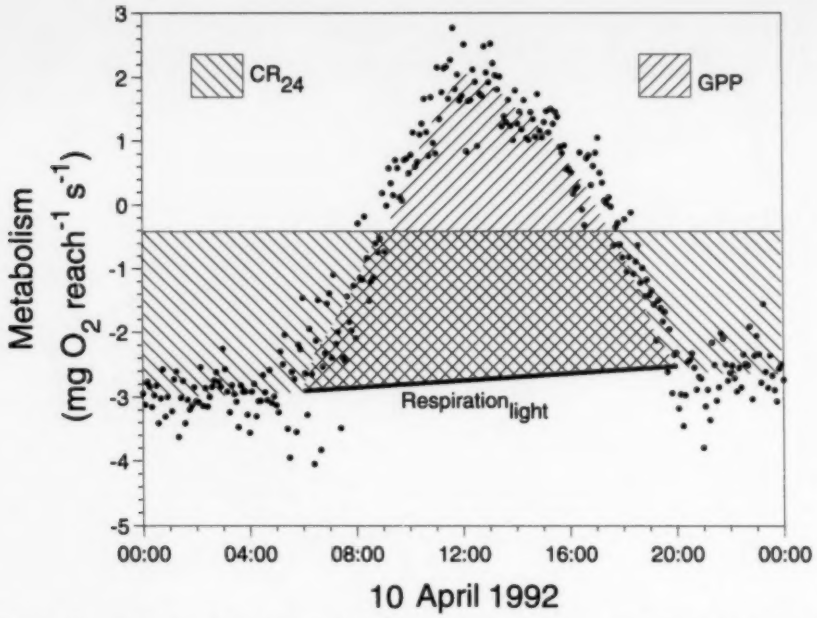

FIG. 2. Metabolism data for 10 April 1992. Gross primary productivity (GPP) is calculated as the difference between the metabolism data and the line estimating respiration$_{light}$. Community respiration (CR_{24}) is the sum of the region indicated by the slashed lines.

our study reach, reaeration flux can be as large or larger than metabolic fluxes of oxygen. Therefore, it is essential to accurately estimate the RC by direct measurements when using the diurnal upstream–downstream oxygen method to determine metabolism in small streams. Genereux and Hemond (1992) have shown that RCs predicted from physical characteristics (gradient, stream velocity, water depth) were generally much lower than direct measurements using the propane technique in Walker Branch. Further, the RCs determined from the propane injections of Genereux and Hemond (1992) were similar to those determined previously from injections of SF_6 in Walker Branch (Wanninkhof et al. 1990), giving us confidence in the propane injection method. Wanninkhof et al. (1990) showed that the gas exchange coefficients measured in Walker Branch were much higher than those measured using the gas injection method in larger streams and rivers and attributed this primarily to the shallow depth and increased surface turbulence.

A variety of predictive equations have been formulated to estimate the RC for a particular reach. The estimates in Table 1 were made using data from Walker Branch for three dates in 1992. None of the equations is well suited to estimate RC for our reach, although the Bennett and Rathbun (1972) equation is within 15% of the measured value on all three dates. Their equation was the only one that did not substantially underestimate the RC on all three dates. The average error of all the predictive equations for all three dates was 70%.

The RC is, in theory, dependent on water temperature (Elmore and West 1961), and the RC needs to be corrected to account for differences in temperature between the time when the RC and oxygen concentration changes were measured. This relationship is $K_2,T1 = K_2,T2 \times 1.0241^{\Delta T}$ where ΔT is the above-mentioned difference in temperature and $T1$ and $T2$ are the actual temperatures. In Walker Branch, diurnal variations in temperature are usually 1–3°C, and therefore the maximum temperature difference should only be a few degrees. For this reason, RC was assumed to be constant in our calculations although it could easily be entered as a temperature-dependent variable for streams with larger diurnal temperature changes.

To calculate the reaeration flux, one must also know the partial pressure of oxygen at the study site and measure temperature continuously throughout the study period for accurate calculations of $DO_{saturation}$ and the oxygen flux. This can be accomplished by correcting for site elevation using equations or by actual atmospheric pressure measurements.

Physical Conditions

Both travel time and RC were strongly affected by autumn leaf-fall because the large abundance of leaves floating on the water surface reduced both gas exchange and water velocity. This was reflected in the much longer travel time and lower RC in November compared with April and July (Table 2). Genereux and Hemond (1992) also observed lower RCs and longer travel times during autumn after leaf-fall in Walker Branch.

Lateral inflows within the reach were low, ranging from 4 to 9% (Table 2). DO measurements of visible inflows were within the range found in the study reach, and therefore, their exclusion from the metabolism calculations was not thought to result in appreciable error. Total daily irradiance to the stream reaches a maximum during the spring (mid-April), prior to canopy closure (W.R. Hill, Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, TN 37831, unpublished data; Rosemond 1994). During the fall and winter, short daylength reduced the total light available for photosynthesis whereas in summer the forest canopy completely

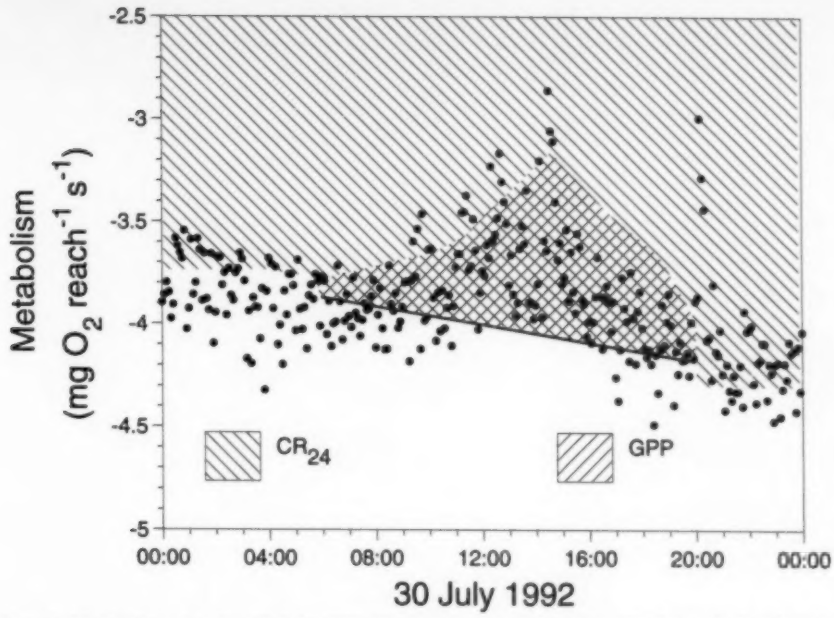

FIG. 3. Metabolism data for 30 July 1992. Gross primary productivity (GPP) is calculated as the difference between the metabolism data and the line estimating respiration$_{light}$. Community respiration (CR_{24}) is the sum of the region indicated by the slashed lines.

shaded the stream, reducing the PAR to <50 $\mu E \cdot m^{-2} \cdot s^{-1}$. Water temperatures during the year ranged from 9 to 17°C within the study reach.

Metabolism

Metabolism in Walker Branch was dominated by respiration (Table 2), not surprising for a small stream in a forested watershed. On two of the dates, photosynthetic oxygen production was insufficient to yield a net export of oxygen from the reach during any portion of the day. On 10 April, photosynthetic oxygen production exceeded respiratory demand during much of the day (Fig. 2). However, even on this date prior to the beginning of forest canopy leafout when irraddiance values were highest, CR_{24} exceeded the total daily GPP (Table 2). In July, when light was uniformly low, the reach consumed more oxygen than it produced at all times of the day (Fig. 3). GPP was almost 17-fold less than on 10 April, while light decreased by about 18-fold (W.R. Hill, Oak Ridge National Laboratory, P.O. Box 2008, Oak Ridge, TN 37831, unpublished data).

Chamber measurements of GPP made on 13–14 April using ceramic tiles from Walker Branch were approximately 20% less than values measured using the whole-stream technique (Table 3). The similarity of GPP values from the chambers and the whole-stream measurements may be misleading, however, because chamber measurements were made during the middle of the day when light and GPP were at a maximum, and the whole-stream value was the average over an entire day. Also, water temperatures in the chambers were about 5°C higher than those for the whole-stream measurements and this may have increased metabolism in the chambers artificially. Assuming that the chamber measurements were indeed overestimates of the daily average, the metabolic measurements made with our whole-stream technique were considerably higher than those made using chambers. The greater whole-stream measurements in April may be due to the presence of bryophytes and seasonally important macroalgae (e.g. *Batrachospermum*) in Walker Branch. These plants, and the leafy liverwort *Porella pinnata* in particular, are conspicuous and likely contribute significantly to GPP in Walker Branch (see below, Steinman and Boston 1993). However, they were not present on the tiles used in the chambers.

The substantially lower respiration values in the chambers, compared with the whole stream, were probably a result of two factors. Macrofauna were absent from the chambers, while their respiratory contribution was included in the whole-stream technique. Second and probably more importantly, the chambers did not include the organic sediments (both surface and hyporheic) which are likely to contain significant respiratory activity. Grimm and Fisher (1984) found that measurements made using in situ open-bottom chambers underestimated respiration compared with whole-stream measurements because their chambers included only surficial sediments (i.e., they did not include the contributions of the deep sediments). Agreement between the two measurements improved when they added measurements made on deep sediment cores to those in the chambers.

GPP not surprisingly was related to light, with highest values in spring prior to forest canopy closure. In summer, both light and GPP were quite low. The GPP measurement made after autumn leaf-fall showed a slight increase over the July rate; however, it was still much lower than in April. This increase in GPP from summer to autumn may reflect decreased light interception by the forest canopy; however, much of the stream bottom remained shaded due to fallen leaves on the stream bottom.

The highest value of CR_{24} occurred on the July date

TABLE 3. Comparison of metabolism values ($\mu g\ O_2 \cdot m^{-2} \cdot s^{-1}$) from in ecos whole-stream diurnal versus in situ chamber measurements. Chamber values are the mean ± SE of six sites along the reach, each of which contained a thalweg and backwater area. Three sites were measured on the 13 and 14 April. For comparison between methods, unit conversion was done with area of the reach as planar, while chamber measurements used the surface area of the sides and one end of the tiles used in each chamber.

	Whole-stream diel 10 Apr. 1992	Chamber (13–14 Apr. 1992)	
		Thalweg	Backwater
Temperature (°C)	13.6	18.5	18.7
GPP	21.45	18.69 ± 1.74	13.38 ± 1.82
CR_{24}	−12.43	−4.15 ± 0.73	−3.39 ± 0.52

(Table 2). Community respiration appears to be influenced by stream water temperature because the highest CR_{24} measurement occurred while the stream water was warmest (Table 2). This is consistent with the results of Rand et al. (1992) who also found whole-stream CR_{24} to be largely influenced by temperature. It is somewhat surprising that the CR_{24} measurement was not higher in November, due to metabolism of microbes associated with the large mass of fallen leaves in the stream at that time. Microbial colonization and growth on the decomposing leaves causes increased phosphorus uptake rates and shorter spiralling lengths during late autumn (Mulholland et al. 1985, 1990; Mulholland 1992). The low CR_{24} measurement and short spiralling lengths may reflect a decoupling of microbial respiration and production, resulting from increased metabolic efficiency. The low CR_{24} could possibly also be the result of direct exchange of oxygen between the atmosphere and wetted leaves on the water's surface, bypassing the flowing water. This process would not be detectable by measurements of DO in the flowing water at the ends of the reach and therefore would not be accounted for by this methodology. Macrofauna respiration probably represents <5% of CR_{24} at all times, based on respirometer measurements of *Elimia* (L. Williams, University of South Carolina, unpublished data) and the fact that *Elimia* represents >95% of the invertebrate ash-free dry mass in Walker Branch (Newbold et al. 1983).

Conclusions

This modified upstream–downstream diurnal oxygen change technique allows whole-stream metabolism measurements to be made on a small stream in which reaeration flux is a major factor determining instream DO concentrations. By determining reaeration coefficients directly through the combined use of conservative and volatile tracers, it is possible to partition upstream–downstream DO changes into reaeration fluxes and metabolism with greater certainty than in the past. Automated DO measurements with high-precision instruments make possible the acquisition of high temporal resolution data. Thus, in ecos whole-stream metabolism measurements can be made accurately without disrupting the study reach. These measurements provide an integrated measure of stream primary productivity and respiration over an entire day that includes all components of the stream. The whole-stream approach is superior to approaches using small samples of substrata and closed chambers for measuring GPP because the latter usually do not include the contributions of patchily distributed macroalgae, bryophytes, and vascular plants. Whole-stream metabolism measurements should be useful in conjunction with other large-scale measurements made on stream reaches, such as nutrient spiralling, elemental mass balances, and secondary productivity.

Based on the whole-stream metabolism data, GPP in Walker Branch is highest during the spring and much lower in summer and autumn, presumably because of reduced light levels after forest canopy closure. Community respiration exceeded GPP on all dates and was highest in July.

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Leeches (Annelida: Hirudinea) in the Experimental Lakes Area, Northwestern Ontario, Canada: Patterns of Species Composition in Relation to Environment[1]

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Grantham, B.A., and B.J. Hann. 1994. Leeches (Annelida: Hirudinea) in the Experimental Lakes Area, northwestern Ontario, Canada: patterns of species composition in relation to environment. Can. J. Fish. Aquat. Sci. 51: 1600–1607.

Canonical correspondence analysis (CCA) was used to examine the distribution of leeches (Annelida: Hirudinea) in 18 lakes at the Experimental Lakes Area (ELA). Leech community composition was best described by an ordination incorporating alkalinity, primary productivity, and lake area. In general, highest species richness occurred in small, eutrophic lakes whereas lowest richness was recorded in medium to large lakes with low productivity. Contrary to results for some other taxa, lake pH was not a dominant variable, describing only a small amount of variance in the species–environment relationship.

L'analyse de corrélation canonique (ACC) a été utilisée pour étudier la répartition de la sangsue (Annelida : Hirudinae) dans 18 lacs de la régions des lacs expérimentaux (ELA). La description de la composition des communautés de sangsues se fait par une ordination tenant compte de l'alcalinité, de la productivité primaire et de l'aire de surface du lac. En général, la richesse en espèces la plus élevée s'observe dans les petits lacs eutrophes, tandis que la diversité la plus réduite est observée dans les lacs de taile moyenne à importante dont la productivité est faible. Contrairement aux résultats obtenus pour d'autres taxons, le pH du lac ne semble pas constituer une variable dominante et ne correspond qu'à une faible fraction de la variance de la relation espèces–environnement.

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Relationships between environmental variables and macroinvertebrate communities have been studied using a variety of approaches, from the predominantly descriptive to univariate and multivariate statistical techniques. Early research on leech (Annelida: Hirudinea) community composition correlated species distributions in lakes with a suite of environmental variables (Mann 1955; Herrmann 1970; Young and Ironmonger 1981), but recently, studies have focused on the relationship between leech occurrence and pH, particularly with respect to the utility of leeches as indicator organisms in acidification studies (Eilers et al. 1984; Mills and Schindler 1986; Schindler et al. 1989; Minns et al. 1990; Bendell and McNicol 1991). These studies have suggested that leeches may be good indicators of acidification, but the relative importance of other environmental factors in determining leech distribution has not been assessed. Patterns of occurrence of several other taxa (molluscs, fish, rotifers) suggest that pH is often not as significant as other environmental variables (Pip 1987; Matuszek and Beggs 1988; Siegfried et al. 1989).

The purpose of this study was to use simple, easily deployed traps to survey a subset of the leech community in a series of lakes to test the hypothesis that leeches are good indicators of lake acidity. This was done by (a) describing the leech fauna in a suite of 18 softwater lakes in the Experimental Lakes Area (ELA) in northwestern Ontario, (b) determining which environmental variables are correlated with changes in leech community composition, and (c) characterizing lake types with respect to their leech fauna. Canonical correspondence analysis (CCA) was used to analyze the influences of various biotic and abiotic factors on the occurrence of leeches.

Study Lakes

The ELA lakes in this study are all softwater lakes on the Canadian Shield. Several lakes have been sites of whole-lake experimental perturbations (reviewed by Schindler 1988); details pertinent to our study are summarized in Table 1.

Methods

Leeches were collected in July 1989 and in June and July 1992 from 18 lakes in the ELA. Activity traps (Murkin et al. 1983), each consisting of a 1-L glass jar with a funnel (stem diameter 1 cm) extending into the jar and held in place by an elastic retainer, were used to sample the leeches. At each lake, five traps baited with pork liver were placed along the shoreline at a depth of 0.3–0.5 m. Traps were set in the late afternoon or evening and retrieved the following morn-

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TABLE 1. Whole-lake manipulations in ELA lakes (modified from Schindler 1988).

Lake	Years	Additions
Acidification		
L223	1976–86	H_2SO_4
L302S	1982–91	H_2SO_4
L302N	1982–86	HNO_3
	1986–88	HCl
L114	1979–86	H_2SO_4
	1984	Al_2SO_4
Eutrophication		
L227	1969–86	P, N, C
L226NE	1973–80	P, N, C
L226SW	1973–80	N, C
L304	1971–72	P, N, C
	1973–74	N, C
	1975–76	P, N
L303	1975–76	P, N
Food-web manipulation		
L221	1987	Pike additions
L222	1987	Pike removals
Heavy metals		
L382	1987–91	Cd additions
Radioactive materials		
L227	1970	Ra^{226}
	1975	H^3
	1973	P^{32}
L224	1976	Ra^{226}, C^{14}, H^3, Se^{75}, Hg^{203}, Cs^{134}, Fe^{59}, Zn^{65}, Co^{60}
L226NE and L226SW	1977	Se^{75}, Hg^{203}, Cs^{134}, Fe^{59}, Zn^{65}, Co^{60}, Sr^{89}

ing. Sampling effort was equivalent for all lakes. Although the sampling method does not adequately sample all leeches, it provides a measure of relative abundance for comparison among lakes and with previous studies (Bendell and McNicol 1991). Leeches captured were transferred to plastic bottles and transported on ice to the laboratory where they were narcotized by slowly dripping 85% ethanol into the water. Specimens were then sorted, counted, preserved in 70% ethanol, and identified according to keys of Davies (1971) and Klemm (1972). Nomenclature follows that of Madill (1985). Identifications of voucher specimens were confirmed by R.W. Davies, University of Calgary, and J. Madill, National Museum of Nature, Ottawa.

Additional information on leech species occurrences was obtained from fish trap-net records (K. Mills and S. Chalanchuk, unpublished data) from many of the same lakes. These records came from sampling periods (May–June, September–October), complementary to those in this study, but added no species to our list.

Eleven morphometric and environmental variables were examined for each lake (Table 2). Morphometric data, including lake area, maximum depth, mean depth, lake volume to 2 m, and shoreline development (calculated from the previous parameters), were obtained from bathymetric maps. Information relating to fish species occurrence was supplied by K. Mills, water chemistry data by M. Stainton, and primary productivity data by E. DeBruyn from ELA long-term databases located at Freshwater Institute, Department of Fisheries and Oceans, Winnipeg. Mean values for July and August 1988–89 were used for pH, specific conductance, dissolved organic carbon (DOC), and alkalinity. July–August means were used because complete open-water data did not exist for several lakes. An examination of open-water period means for those lakes with more complete records produced no difference in the ranking of lakes with respect to these variables. Mean annual primary productivity values for 1988–89 were based on morphometrically corrected epilimnion samples. Two-year means for the chemical and primary productivity data were utilized because several of the lakes have extensive perturbation histories and thus long-term means would not reflect current limnological conditions.

Species and environmental data were analyzed using a direct gradient analysis technique, CCA developed by ter Braak (1986) and included as a procedure in CANOCO (version 3.12, ter Braak 1989). CCA is a constrained ordination technique in which the unimodal response of species to environmental variables is incorporated. Linear combinations of environmental variables are selected to produce maximum separation of the species' distributions. The CCA procedure produces an ordination diagram in which species and sites are represented by points and environmental variables are represented by vectors. The longer the vector, the greater the correlation between the variable and the ordination axes, and hence the greater that variable's influence on the pattern of community variation (ter Braak 1986).

For this analysis, the chemical and morphometric vari-

TABLE 2. Characteristics of ELA lakes sampled in this study. Primary productivity and chemical data consist of 1988–89 epilimnetic means except where specified. Fish spp. = number of fish species, Prod. = primary productivity, Cond. = conductivity, Alk. = alkalinity, DOC = dissolved organic carbon, Shore = shoreline development, Z_{max} = maximum depth, Vol. = volume to 2 m, and Z_{mean} = mean depth.

Lake	Fish spp.	Prod. (mg C·m^{-3})	n	pH	Range	Cond. (μS·cm^{-1})	Range	Alk. (μeq·L^{-1})	Range	DOC (μmol·L^{-1})	Range	Shore	Area (ha)	Z_{max} (m)	Volume to 2 m	Z_{mean} (m)
L114	4	22.23[a]	9	6.47	6.18–6.85	20.56	19–22	43.75[b]	31–61	623.33	570–690	1.89	12.10	5.0	1.818×10^5	1.71
L221	3	9.40	4	6.70	6.47–6.87	25.50	25–26	92.50	75–105	1022.50	980–1150	1.45	9.03	5.7	1.350×10^5	2.09
L222	5	6.45	6	7.16	7.00–7.37	33.67	32–37	216.33	192–239	850.00	700–930	1.44	16.39	5.8	2.910×10^5	3.66
L223	4	7 19	15	5.85	5.68–6.03	33.89	32–39	4.61	1–15	282.78	240–440	1.36	27.27	14.4	5.080×10^5	7.15
L224	6	4.00	8	7.09	6.98–7.33	20.88	20–23	82.50	76–88	230.00	210–250	1.53	25.92	27.4	4.500×10^5	11.59
L225	0	12.83[c]	10	4.96	4.82–5.09	23.50	20–25	5.20	1–12	883.00	700–1050	1.62	3.99	2.0	4.710×10^4	1.18
L226	6	7.54	13	6.98	6.90–7.11	21.86	21–26	98.00	90–110	541.43	490–610	2.21	16.10	14.7	2.994×10^5	5.97
L227	5	64.49	18	9.36	8.84–9.97	33.33	30–36	187.56	125–231	1419.44	1140–1680	1.14	5.00	10.0	8.310×10^4	4.42
L239	7	4.99	8	7.29	7.14–7.57	34.88	34–37	161.25	151–168	517.14	460–580	1.63	56.10	30.4	1.040×10^6	10.53
L240	4	6.24	9	7.29	7.08–7.56	32.11	30–34	143.22	135–152	511.11	440–570	1.31	44.10	13.1	8.090×10^5	6.05
L302N	7	12.62	18	5.22	5.00–5.61	33.83	32–35	6.89	1–41	441.11	350–640	1.63	12.80	13.8	2.290×10^5	5.72
L302S	2	9.48	18	4.55	4.44–4.74	47.22	41–53	−30.89	−12–50	174.44	70–390	1.82	10.90	10.6	2.001×10^5	5.08
L303	4	32.87[d]	9	6.80	6.51–7.06	23.00	21–25	78.78	65–89	588.89	520–670	1.47	9.93	2.5	1.466×10^5	1.51
L304	0	14.38	8	6.84	6.68–7.08	23.50	23–25	98.00	88–110	787.50	740–880	1.38	3.62	6.7	6.190×10^4	3.18
L375	6	5.10	5	7.63	7.52–7.70	50.00	47–54	359.40	333–394	428.00	390–470	1.46	18.73	26.0	3.390×10^5	11.61
L382	6	7.12	9	7.04	6.81–7.17	25.00	24–27	101.44	95–108	606.67	510–690	1.69	36.86	13.0	6.520×10^5	5.77
L470[e]	3	7.00[c]	8	6.13	5.47–6.56	27.38	21–39	88.38	45–134	1321.25	990–1760	1.18	4.24	1.7	3.330×10^4	0.79
L661[g]	4	8.70[h]	17	6.07	5.74–6.43	27.88	21–34	55.12	35–78	1546.00[i]	1020–1870	1.40	2.00	1.5		

[a] 1988 data.
[b] $n = 8$.
[c] 1984–86 mean.
[d] 1984–86 mean.
[e] Outflow data.
[f] 1975 data.
[g] Volume to 2 m and Z_{mean} estimated.
[h] 1982 data.
[i] $n = 10$.

TABLE 3. Leech community composition in 18 lakes at ELA.

	Lake (pH)																		
	L302S (4.55)	L225 (4.96)	L302N (5.22)	L223 (5.85)	L661 (6.07)	L470 (6.13)	L114 (6.45)	L221 (6.7)	L303 (6.8)	L304 (6.83)	L226 (6.98)	L382 (7.04)	L224 (7.09)	L222 (7.16)	L239 (7.29)	L240 (7.29)	L375 (7.63)	L227 (9.36)	Range
Hirudinidae																			
Mollibdella grandis						+				+	+		+	+	+	+			6.13–7.29
Macrobdella decora	+	+			+	+	+		+	+	+	+	+	+			+	+	4.55–9.36
Percymoorensis marmoratis	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	4.55–9.36
Erpobdellidae																			
Erpobdella punctata						+	+					+						+	6.13–9.36
Nephelopsis obscura	+		+		+	+	+	+	+	+	+	+	+	+	+			+	4.55–9.36
Glossiphoniidae																			
Placobdella spp.	+		+		+	+	+			+	+		+	+			+	+	4.55–9.36
Glossiphonia complanata			+		+	+			+	+	+							+	5.22–9.36
Helobdella stagnalis	+		+	+		+				+	+								4.55–6.98
Number of species	5	2	5	2	5	8	5	1	4	7	7	4	5	5	3	2	3	6	

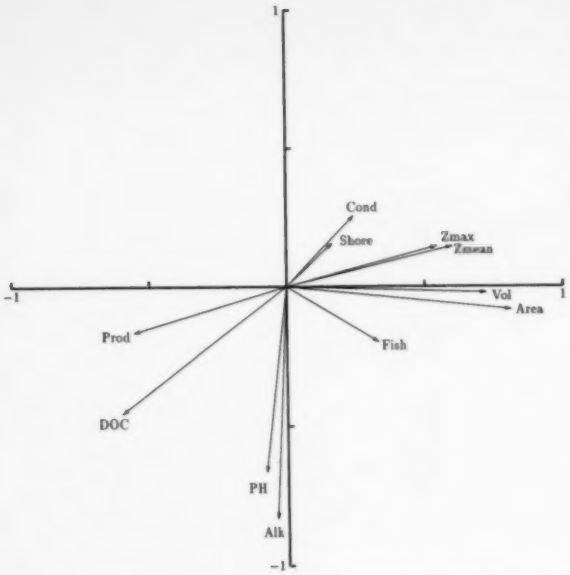

FIG. 1. CCA ordination showing all environmental variables (arrows). The first ordination axis is horizontal and the second is vertical. Arrows point in the direction of maximum change of the variable, and arrow length indicates the strength of correlation between the variable and the ordination axis. Variables with arrows that form an acute angle are inferred to be positively correlated and with an obtuse angle to be negatively correlated. Variables correspond to those listed in Table 2.

ables (except pH and shoreline development) were log transformed, and species numbers were square-root transformed to more closely approach a normal distribution. Three species of *Placobdella* (*P. ornata*, *P. parasitica*, *P. papillifera*) were pooled (*Placobdella* spp.) and treated as a passive sample due to their low numbers in all lakes. Passive samples appear on the ordination diagrams, but do not influence determination of the ordination axes. In the initial analysis, all environmental variables were included; however, because many of these were highly correlated, partial CCA using forward selection (analogous to the selection process in stepwise multiple regression) was performed to reduce the number of variables to those that contribute significantly to explaining total variance of the species data (ter Braak 1987). The significance of each variable added, as well as the first canonical axis, was tested ($P < 0.05$) using a Monte Carlo permutation test.

Results

Ten species of leeches representing three families were collected during the study (Table 3). Leeches were found in all lakes surveyed, representing a pH range of 4.55–9.36. The overall mean species richness in 18 lakes was 4.4 ± 0.45, with a range of one to eight species and modal species richness of six species. The mean number of species captured from lakes with pH > 6.0 (4.6 ± 0.51) exceeded that from lakes with pH < 6.0 (3.5 ± 0.75), but this difference was not significant (Mann–Whitney test, $P > 0.05$). The relationship between leech species richness and pH in these lakes was not significant (Pearson product-moment correlation, $P > 0.05$). The highest species richness occurred in the smaller, more eutrophic lakes (L470, L304, L227, L226). *Percymoorensis marmoratis* was the most commonly captured species, occurring in 94% of lakes. *Nephelopsis obscura* was found in 78% of lakes and *Macrobdella decora* in 72% of lakes.

In our CCA, no single variable exhibited a predominant influence on leech abundance and distribution (Fig. 1). The first ordination axis is explained mainly by area, volume, and primary productivity and the second by alkalinity and pH. Of the environmental variables used, volume, maximum depth, mean depth, DOC, and fish species richness were correlated with area whereas pH was correlated with alkalinity (Table 4).

Forward selection revealed that alkalinity, primary productivity, and area explained significant proportions of the variance in the species data. In the triplot of species, lakes, and the selected environmental variables (Fig. 2), the first two ordination axes explain 22.1 and 14.2% of the variance in the species data and 55.0 and 35.1% of the variance in the species–environment relationship. Species are significantly related to environmental variables (F-ratio = 3.71, $P = 0.03$). Area ($r = 0.77$) and primary productivity ($r = -0.57$) were correlated with the first ordination axis and alkalinity ($r = -0.77$) with the second axis.

The ordination of lakes on the first two axes (Fig. 2) showed three clusters: low-pH lakes (L225, L302S, L302N, L223), large, oligomesotrophic lakes (L239, L240, L382, L224, L226, L375, L222), and smaller, eutrophic lakes (L661, L470, L304, L303, L227, L221, L114). The distribution of leech species reveals a less clear-cut pattern. *Erpobdella punctata*, *M. decora*, *N. obscura*, and *Glossiphonia complanata* tend to occur in the smaller, more productive lakes. *Helobdella stagnalis* is associated with the moderately acidic, mesotrophic lakes, and *P. marmoratis* and *Mollibdella grandis* are found in the larger, oligotrophic lakes. *Placobdella* spp. display no strong association to a particular lake type. In the three clusters of lakes delineated by the ordination, *P. marmoratis* was the dominant species in the acidic lakes as well as in the larger, oligotrophic lakes. *Nephelopsis obscura* was dominant in the smaller, eutrophic lakes.

Discussion

Relationship between Environmental Variables and Species Distributions

CCA detects the variation in species distributions that is explained by the chosen environmental variables, and thus allows the establishment of an environmental basis for observed patterns of community composition. The distributions of the eight taxa (comprising 10 species) collected from these lakes are governed primarily by two gradients represented by the axes in the ordination triplot (Fig. 2): a trophic–areal axis extending from small, eutrophic lakes on the left to large, oligomesotrophic lakes on the right and an alkalinity axis representing acidic lakes with low alkalinity at the upper right to higher alkalinity lakes at the lower left.

The areal portion of the trophic–areal gradient represents several intercorrelated parameters, including DOC, lake volume, shoreline development, and maximum and mean depths. With the exception of DOC, all of these variables influence the ratio of littoral and pelagic volumes and hence the availability of suitable littoral habitat. In this study, highest

TABLE 4. Correlation matrix of environmental variables.

	Fish spp.	Prod.	pH	Cond.	Alk.	DOC	Shore	Area	Z_{max}	Vol.	Z_{mean}
Fish spp.	1.0000										
Prod.	−0.1555	1.0000									
pH	0.2236	0.4449	1.0000								
Cond.	0.1719	−0.0194	0.0706	1.0000							
Alk.	0.1476	−0.0101	0.7467	−0.0933	1.0000						
DOC	−0.2016	0.3398	0.2243	−0.0134	0.3923	1.0000					
Shore	0.2946	−0.2156	−0.1863	−0.3961	−0.2079	−0.5361	1.0000				
Area	0.5476	−0.3712	−0.1348	0.0585	0.0696	−0.7241	0.4720	1.0000			
Z_{max}	0.3582	−0.1453	0.3717	0.1489	0.0753	−0.7172	0.4522	0.7603	1.0000		
Vol.	0.4370	−0.2322	0.2176	0.0567	0.0642	−0.7534	0.4888	0.9702	0.8387	1.0000	
Z_{mean}	0.3565	−0.2024	0.3666	0.2062	0.0972	−0.7301	0.3881	0.7727	0.9875	0.8456	1.0000

species richness was recorded in small lakes with high ratios of littoral to pelagic area.

Fish species richness, closely correlated with the areal variables, particularly volume, was not a strong factor influencing establishment of the ordination axes. However, it appears that intensity of fish predation may exert a strong influence on the leech community in some ELA lakes. For example, seven of eight leech taxa in the regional species pool were recovered in L304, where fish were extirpated by an experimental manipulation and winterkills. Only L470, a small, shallow lake that also periodically winterkills, contained a greater diversity of leeches. Although speculative, the anomalously low leech diversity in L221 may also be a result of increased predation pressure from the introduced pike population. The relationship between number of fish species and leech species richness was not significant in our study ($P > 0.05$), probably because the other lakes with reduced fish fauna were also highly acidic lakes with depauperate leech faunas.

The trophic gradient reflects the underlying influence of lake productivity (as represented by primary productivity) on leech distribution. Sawyer (1974) suggested that food supply is the primary limiting resource governing leech distribution and that leeches should be most abundant in eutrophic environments. In our study, eutrophic lakes generally supported higher leech diversity than did oligotrophic lakes. This may result from the increased availability of chironomid larvae and other invertebrate taxa whose biomass in ELA lakes is correlated with increasing primary productivity (Davies 1980). Species richness has also been found to reflect trophic status in freshwater gastropods (Pip 1987) and leeches (Herrmann 1970).

The alkalinity gradient, including pH, was not a strong factor governing leech distribution at ELA, except at extremely low pH values. Four of the lakes in our study had extremely low alkalinities (<7 eq·L^{-1}), yet in two of these, we captured five leech species. Although Mann (1955) found a relationship between total alkalinity and leech community composition in Britain, most other studies report that leech distribution is not affected by alkalinity (Herrmann 1970; Young and Ironmonger 1982).

Alkalinity and pH may exert indirect effects on leeches via prey quantity and quality. Those leech species with specialized diets, e.g., molluscivores, would be more severely impacted by low alkalinity and pH than would omnivorous species. *Glossiphonia complanata* and the gastropods on which it primarily feeds (Wrona et al. 1979) are both absent from lakes with pH < 5.0.

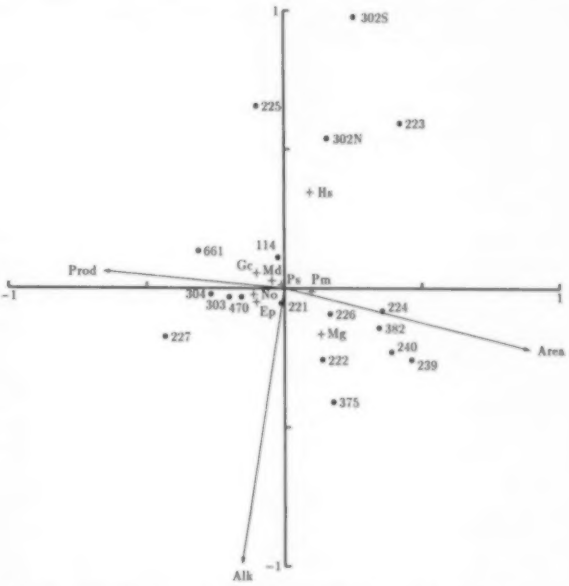

FIG. 2. Distribution of leeches in 18 lakes at the ELA, northwestern Ontario. CCA ordination diagram based on forward selection of three environmental variables. Leech species are indicated by crosses, lakes by solid circles, and environmental variables by arrows.

Community Composition

Percymoorensis marmoratis and *M. grandis* characterize the leech fauna of moderate to large oligotrophic lakes at ELA, as was the case in Bendell and McNicol's (1991) survey of Ontario lakes. *Nephelopsis obscura*, *Erpobdella punctata*, *M. decora*, and *G. complanata* typify the leech community in the small, slightly dystrophic to eutrophic Shield lakes. *Erpobdella punctata*, consistently associated with organically polluted sites, probably because of increased prey availability (Barton and Metcalfe 1986), occurred in only a few of the more eutrophic lakes in our study. *Glossiphonia complanata* has been recorded most frequently from lakes with stony bottoms and high Ca content, and previous records suggest that it does not occur in dystrophic or acidotrophic lakes (Sawyer 1974); however, in this study, it occurred at pH 5.22. *Macrobdella decora*, found widely throughout North America (Moore 1923), occurred in great-

est abundance in small, eutrophic Shield lakes (L470, L226, L661). *Helobdella stagnalis* is a widely distributed species and has been found in lakes with pH < 5.1 (Bennike 1943; Raddum and Fjellheim 1984). In this study, it occurred most frequently in circumneutral to moderately acidic lakes. The occurrence of the parasitic *Placobdella* spp. is most likely related to host availability, as is the case in Colorado where the distributions of *P. parasitica* and *P. ornata* parallel those of their turtle hosts (Herrmann 1970). However, numbers of each species were too small for any definitive correlations.

Acidification Effects

Leeches were collected from a wider range of pH values in this study than in any previous study of leech distribution (Table 3) (Eilers et al. 1984; Raddum and Fjellheim 1984). Although Bendell and McNicol (1991) found no leeches in lakes with pH < 4.9, we found five species occurring at pH 4.55 (L302S). One of these species, *P. marmoratis*, was also found by Bendell and McNicol (1991) at pH 4.9; *H. stagnalis* has been found at pH 4.0 (Bennike 1943). The median of minimum pH values for the eight leech taxa in our study approached pH 4.5, considerably lower than the pH 6.6 reported by Eilers et al. (1984) in a synoptic literature survey, but similar to the pH 5.2 determined by Bendell and McNicol (1991).

Several caveats need to be considered in evaluating these observations. The data from the experimentally acidified lakes must be interpreted carefully because in these whole-lake experiments, only the lake itself was treated with acid whereas the entire watershed is affected by anthropogenic acidification, resulting in the leaching from soils, and possibly increased mobilization, of heavy metals (Stokes et al. 1989). Synergistic interactions between these metals and low pH could result in leeches being eliminated at higher pH values than during experimental lake acidification. A second consideration is that the pH values cited here are based on epilimnetic samples whereas littoral and (or) benthic pH values may be somewhat higher than indicated by open-water readings (Kelly et al. 1984; Dermott 1985). In addition, the presence of nonacidified inflows into experimentally acidified lakes may produce enclaves of moderate pH in which populations of some species of leeches can survive and reproduce.

Specialization and sensitivity to the environmental contaminant of concern are two important attributes of an indicator species (Landres et al. 1988). Most of the species examined in this study are generalist omnivores whose diets change with food availability (Sawyer 1986; B.A. Grantham, unpublished data), making them relatively insensitive to fluctuations in prey species and thus reducing their usefulness as indicators of environmental change. In addition, the pH ranges of the species observed in this study indicate that leeches are not particularly good indicators of acidification, especially as "early warning" taxa, since their distribution is affected by pH only at extremely low values. Above this threshold, the dominant leech taxa occurred in almost every lake, and fluctuations in leech species richness were not correlated with pH. A similar lack of sensitivity was evident for the other variables examined in this study. Thus, we conclude that leeches, although demonstrating reductions in species diversity at very low pH levels, are poor indicators of lake acidification.

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Mitochondrial DNA Variation in Introduced Populations of Pacific Oyster, *Crassostrea gigas*, in British Columbia

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Boom, J.D.G., E.G. Boulding, and A.T. Beckenbach. 1994. Mitochondrial DNA variation in introduced populations of Pacific oyster, *Crassostrea gigas*, in British Columbia. Can. J. Fish. Aquat. Sci. 51: 1608–1614.

Crassostrea gigas, a species native to Japan, was transplanted in very large numbers from the Miyagi Prefecture from 1927 to 1977 to British Columbia coastal waters. We examined the genetic structure and diversity of wild British Columbian populations of *C. gigas* using restriction enzyme analysis of mitochondrial DNA. Forty-four mtDNA haplotypes were identified in 141 animals from the populations sampled, implying the existence of considerable genetic diversity. The frequency distribution of these haplotypes is unusual in that only two haplotypes predominate whereas 34 are represented by a single individual and five by two individuals. The four British Columbia populations sampled all shared the two common haplotypes in roughly the same frequencies and a third haplotype at low frequencies. Between 9 and 11 haplotypes were found uniquely in each of the four British Columbia populations sampled, but 34 of those 40 private haplotypes were observed only once. Much larger sample sizes would be required to conclude that those haplotypes are indeed "private" and not present in low frequencies in all populations. We conclude that there is substantial genetic variation in these introduced populations, but there is little evidence of genetic divergence between them.

Crassostrea gigas, une espèce originaire du Japon, a été transplantée en très grand nombre, entre 1927 et 1977, de la préfecture de Miyagi aux eaux côtières de Colombie-Britannique. Nous avons étudié la structure génétique et la diversité des populations sauvages de *C. gigas* en Colombie-Britannique par une analyse de l'ADN mitochondrien à l'aide d'enzymes de restriction. Quarante-quatre haplotypes d'ADNmt ont été identifiés chez 141 spécimens des populations échantillonnées, ce qui indique une diversité génétique considérable. La distribution statistique de ces haplotypes est inhabituelle en ce que seuls deux haplotypes prédominent, tandis que 34 sont représentés par un seul individu et cinq par deux individus. Dans les quatre populations de Colombie-Britannique échantillonnées, on retrouve les deux haplotypes communs à peu près aux mêmes fréquences, ainsi qu'un troisième haplotype à des fréquences plus basses. Entre 9 et 11 haplotypes ont été retrouvés uniquement dans chacune des quatre populations de Colombie-Britannique échantillonnées, mais 34 de ces 40 haplotypes « spécifiques » ont été observés une seule fois. Il faudrait disposer d'échantillons beaucoup plus nombreux pour conclure que ces haplotypes sont réellement « spécifiques » et qu'ils ne sont pas présents à une fréquence très réduite dans toutes les populations. Nous concluons que ces populations introduites sont sujettes à une variation génétique substantielle, mais qu'il n'existe que peu de preuves de divergences génétiques entre elles.

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The Pacific oyster, *Crassostrea gigas*, is a species native to coastal areas of Japan. Its potential as an aquacultural species has led to its introduction to many areas of the world (reviewed by Chew 1990). Four strains of this species are recognized in Japan, each having distinctive morphological features and developmental schedules (Numachi 1978; Quayle 1988). The Hokkaido strain is found in the most northerly distributions, followed by the Miyagi, the Hiroshima, and, at the southernmost extreme, the Kumamoto. Growth and survival of Japanese oysters in British Columbia waters was first explored with the introduction of a few Miyagi animals early in this century, as these were considered best suited to British Columbia conditions (Numachi 1978; Quayle 1988). Promising results in the growth and survival of juveniles and adults led to the importation of very large numbers of Miyagi spat from 1927 to 1977.

The first major spawning of introduced populations occurred in Ladysmith Harbour, on the east coast of Vancouver Island, in 1932 (Quayle 1964). This and two additional spawnings in Ladysmith Harbour in 1936 initiated recruitment in other areas of the Strait of Georgia. Major breeding events in 1942, 1958, and 1961 established several reproducing populations confined to the Strait of Georgia (Quayle 1988). Reproducing populations on the west coast of Vancouver Island were generated with juveniles from the 1936 Ladysmith Harbour spawning. Repeated spawnings of populations on both coasts led to a cessation of spat imports, as it appeared that wild set was sufficient for both widespread recruitment and a reliable source of seed for industry production (Bourne 1978; Quayle 1988).

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Since its introduction, *C. gigas* has reproduced only in those areas where adequate food and temperature requirements are met (Quayle 1964, 1988; Numaichi 1978; Ketchen et al. 1983). Conditions must be adequate not only for full gonadal development and the initiation of spawning, but also to maintain normal developmental rates and larval survival. These conditions have occurred inconsistently over the years, and consequently, recruitment in most areas has been sporadic. Major spawning events of the scope and scale noted above have not occurred in three decades (Quayle 1988). Wild recruitment has become less predictable in recent years and industry now relies almost exclusively on larvae from hatcheries and remote spat setting technology for production (Jones and Jones 1982).

The effect of restricted reproduction on the genetics of British Columbia oyster populations is not known. Hence, we examined the outcome of the genetic history of wild populations of *C. gigas* using restriction enzyme analysis of mitochondrial DNA (mtDNA) (Lansman et al. 1981). The diversity of mtDNA genotypes in populations has proven to be very useful as an indicator of genetic variation and population structure and is particularly sensitive to reproductive restrictions that can impose significant genetic bottlenecks on introduced or reproductively confined species (Bernatchez et al. 1989; Chapman 1989; Sunden and Davis 1991).

Materials and Methods

Four populations for which introduction and spawning histories were well documented were chosen for sampling (Quayle 1964, 1988; Bourne 1978). Each of these has been set aside by the British Columbia Ministry of Agriculture, Fisheries, and Food as spawning populations from which wild seed can be collected by industry; harvesting and other activities associated with oyster culture are strictly regulated. These populations include Pendrell Sound (PN), Hotham Sound (HT), Nootka Sound in Hisnitt Inlet (HS), and Barclay Sound in Pipestem Inlet (PS). Collection localities are shown in Fig. 1. Approximately 150 animals were chosen from each site at random along a transect spanning low to high intertidal areas. These specimens were held at 4–8°C in a self-contained refrigerated saltwater holding facility at Simon Fraser University until a subsample of about 35 individuals could be taken for further processing. The sampling procedure covered all sizes except animals with a shell length less than 7 cm. This restriction was necessary to ensure sufficient mtDNA yields for analysis, but a broad range of year classes (2 yr and older) was sampled. An additional sample from Washington State (DB) was provided by Dr. Dennis Hedgecock of the Bodega Marine Laboratory to represent the 1985 year class of Dabob Bay wild stocks.

To extract mtDNA, the "sucrose mini-gradient" method (Jonathan Terrett, Queen's Medical Centre, University of Nottingham, personal communication) was combined with the mtDNA extraction methods developed for the purification of mtDNA from scallop adductor muscle (Snyder et al. 1987). Briefly, 0.5–5.0 g of tissue was homogenized in 25 mL of isotonic sucrose buffer (0.5 M sucrose, 0.15 M KCl, 2 mM ethylenediaminetetraacetic acid (EDTA, disodium salt), 25 mM *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulphonic acid (HEPES), pH 7.4) using a motor-driven Teflon/glass homogenizer. Protease type XXIII (Sigma) was added to a concentration of 0.2 g/mL and the homogenate incubated

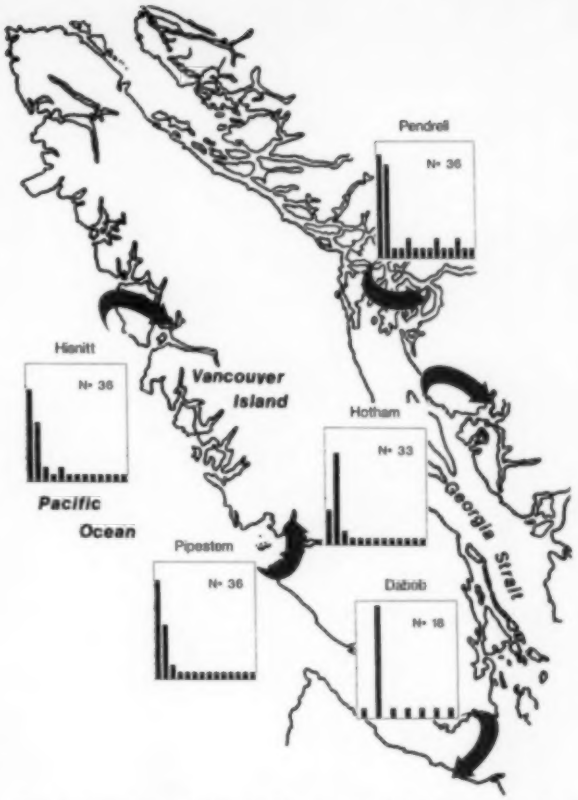

FIG. 1. Locations of the populations sampled and a summary of population genetic structure as revealed by haplotype frequency data (Table 1). The first two bars in each frequency distribution represent the two dominant haplotypes, PS1 and PS2, respectively. Most of the other haplotypes were unique to each population.

at 37°C for 0.5–1.5 h to reduce viscosity. Following centrifugation at 2500 rpm (750*g*) in a Beckman JA-20 rotor for 10 min to remove nuclei and debris, the mitochondria were sedimented from the supernatant in the same rotor at 16 000 rpm (30 000*g*) for 30 min. The mitochondrial pellet was resuspended in 1 mL of isotonic sucrose buffer and layered on top of 0.5 mL of 1.1 M sucrose, 5 mM EDTA, and 10 mM Tris–HCl (pH 7.5) in a microfuge tube. Mitochondria were pelleted through the sucrose at top speed (15 000*g*) for 10 min in a microfuge. The sucrose "mini-gradient" spin was repeated and the purified mitochondria were resuspended in STE100 (100 mM NaCl, 100 mM EDTA, 10 mM Tris–HCl, pH 8.0) and lysed with Nonidet NP-40. Following treatment with 1% cetyltrimethyl-ammonium bromide (CTAB) in the presence of 0.7 M NaCl to remove residual polysaccharides and glycogen, the mtDNA was extracted with chloroform – isoamyl alcohol (24:1) and precipitated with isopropanol at −20°C. The purified mtDNA samples were dissolved in 20 μL of TE (10 mM Tris, 1 mM EDTA, pH 8.0) and held at 4°C until further analysis.

Restriction enzyme digests were performed in 5.0-μL volumes under conditions specified by the manufacturer (Bethesda Research Laboratories). A number of enzymes were tested and those that produced five or more restriction fragments were chosen for this study: *Hpa*1, *Hin*dIII,

TABLE 1. Mitochondrial DNA haplotypes in Pacific oysters. The "A" genotype for restriction enzyme profiles and for haplotype frequencies of zero is denoted by "—" for clarity. Genotype assignments were made in the order in which they were encountered.

	Enzyme[a]									Population frequencies[b]					
	1	2	3	4	5	6	7	8	9	PS	PN	HT	HS	DB	BC
PS1	—	—	—	—	—	—	—	—	—	0.42	0.31	0.15	0.39	0.06	0.32
PS2	—	B	—	—	—	—	—	—	—	0.23	0.28	0.39	0.25	0.67	0.28
PS3	—	—	B	—	—	—	—	—	—	0.06	—	—	0.06	—	0.03
PN4	—	—	—	—	—	B	—	—	—	—	0.03	—	—	—	<0.01
PN5	—	—	—	—	—	C	—	—	—	—	0.03	—	—	—	<0.01
PS6	—	—	D	—	—	—	—	—	—	0.03	—	—	—	—	<0.01
PN7	—	D	—	—	—	—	—	—	—	—	0.06	—	—	—	0.01
PN8	—	—	—	E	—	—	—	—	—	—	0.03	—	—	—	<0.01
PN9	—	—	G	—	—	—	—	—	—	—	0.03	—	—	—	<0.01
PS10	—	—	—	B	—	—	—	—	B	0.03	—	—	—	—	<0.01
PS11	—	—	B	C	—	—	—	—	—	0.03	—	—	—	—	<0.01
PS12	—	—	—	—	B	—	—	C	—	0.03	—	—	—	—	<0.01
PN13	—	E	B	—	—	—	—	—	—	—	0.03	—	—	—	<0.01
PN14	—	—	E	D	—	—	—	—	—	—	0.06	—	—	—	0.01
PS15	—	C	C	—	—	—	—	B	—	0.03	—	—	—	—	<0.01
PS16	B	B	—	—	—	—	B	—	—	0.03	—	—	—	—	<0.01
PN17	—	B	F	—	—	—	—	D	—	—	0.03	—	—	—	<0.01
PS18	—	B	F	—	—	—	—	—	—	0.03	0.03	0.06	0.03	—	0.04
PS19	—	—	—	B	—	—	—	—	—	0.03	—	—	—	—	<0.01
PS20	—	—	—	—	—	—	—	—	F	0.03	—	—	—	—	<0.01
PS21	—	—	—	—	—	—	—	E	—	0.03	—	—	—	—	<0.01
PN22	—	G	—	—	—	—	—	—	—	—	0.06	—	—	—	0.01
PN23	—	B	J	—	—	—	—	—	—	—	0.03	—	—	—	<0.01
PN24	—	B	K	—	—	—	—	—	—	—	0.03	—	—	—	<0.01
HS25	—	B	L	—	—	—	—	—	—	—	—	—	0.06	—	0.01
HS26	—	—	H	—	—	D	—	—	—	—	—	—	0.03	—	<0.01
HS27	B	B	G	—	—	—	B	—	—	—	—	—	0.03	—	<0.01
HS28	—	B	I	—	—	—	—	—	—	—	—	—	0.03	—	<0.01
HS29	—	B	—	F	—	—	—	—	—	—	—	—	0.03	—	<0.01
HT30	C	B	—	—	—	—	—	—	C	—	—	0.03	—	—	<0.01
HT31	—	B	—	G	—	E	—	—	D	—	—	0.03	—	—	<0.01
HT32	—	B	—	—	—	F	—	—	—	—	—	0.09	—	0.06	0.02
HT34	C	—	—	—	—	—	—	—	E	—	—	0.03	—	—	<0.03
HT35	—	B	—	—	—	E	—	—	D	—	—	0.06	—	—	0.01
HT36	—	B	—	—	—	—	—	—	B	—	—	0.03	—	—	<0.01
PS41	—	—	—	—	—	—	—	—	G	0.03	—	—	—	—	<0.01
HS42	—	—	H	—	—	—	—	—	—	—	—	—	0.03	—	<0.01
HS43	—	B	—	J	—	—	—	—	—	—	—	—	0.03	—	<0.01
HS44	—	B	M	—	—	—	—	—	B	—	—	—	0.03	—	<0.01
HS45	—	B	N	F	—	—	—	—	—	—	—	—	0.03	—	<0.01
HT46	—	B	—	—	—	—	—	F	—	—	—	0.03	—	—	<0.01
HT47	—	H	—	—	—	—	—	—	—	—	—	0.03	—	—	<0.01
HT51	—	—	—	—	—	—	—	—	H	—	—	0.03	—	—	<0.01
HT52	—	B	—	—	—	—	—	—	I	—	—	0.03	—	—	<0.01
DB37	—	—	—	H	—	—	—	—	—	—	—	—	—	0.06	—
DB38	—	C	—	—	—	—	—	—	—	—	—	—	—	0.06	—
DB39	—	F	—	—	—	—	—	—	—	—	—	—	—	0.06	—
DB40	—	B	—	I	B	—	—	—	—	—	—	—	—	0.06	—
Sample sizes										36	36	33	36	18	141

[a]1 = *Hpa*1; 2 = *Hin*dIII, 3 = *Hae*III; 4 = *Hpa*II; 5 = *Ava*1; 6 = *Hae*II; 7 = *Hin*cII; 8 = *Ava*II; 9 = *Hin*f1.
[b]PS = Pipestem; PN = Pendrell; HT = Hotham; HS = Hisnitt; DB = Dabob (Washington); BC = total for the four British Columbia populations.

*Hae*III, *Hpa*II, *Ava*1, *Hae*II, *Hin*cII, *Ava*II, and *Hin*f1. This set of enzymes produced a total of 131 restriction sites and sampled approximately 2.5% of the oyster mitochondrial genome.

Following standard agarose gel electrophoresis (Sambrook et al. 1989) in the presence of ethidium bromide (0.5 μg/mL) and photography on a UV transilluminator, gels were processed for Southern transfer to GeneScreen nylon hybridization membranes according to the manufacturer (New England Nuclear). Restriction fragments were crosslinked to the membranes with UV light (Stratagene Cloning Systems) and dried at 80°C. Oyster mtDNA was purified on cesium chloride gradients or isolated as described above and labelled with [^{32}P]dATP by random-primed DNA synthesis with

T7 DNA polymerase (Stratagene Cloning Systems) for use as a probe. Prehybridization and hybridization were carried out in 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM PB (equimolar mono- and dibasic sodium phosphate), 2 mM EDTA), 5× Denhardt's (1× Denhardt's is 0.02% Ficoll type 400, 0.02% bovine serum albumin (fraction V), 0.02% polyvinylpyrolidone (PVP-40), 0.3% sodium dodecyl sulphate (SDS) in a shaking water bath at 65°C. Posthybridization washes were performed at 65°C in 0.5× SSPE, 0.3% SDS. Membranes were autoradiographed using Kodak XK-1 film at room temperature, or with intensifying screens at −70°C, for one to several days. A typical experiment involved a range of exposures to confirm the resolution of closely spaced or small fragments. Fragments down to 200 bp (base pairs) were detected and scored in this study.

Data analyses were carried out using programs in the Restriction Enzyme Analysis Program package (REAP, programs DA and DSE; Doug McElroy, Department of Zoology, Arizona State University, Tempe, AZ 85287-1501). Nucleotide divergence estimates (Nei and Tajima 1981; Nei and Miller 1990) and their respective standard errors (Nei and Tajima 1983) were computed using DSE. Intra- and interpopulational nucleotide diversity and divergence estimates and estimates of nucleon diversity were calculated using DA. UPGMA analysis of distance data produced by REAP was performed using Phylip version 3.4 (Felsenstein 1991).

Results and Discussion

From the digestion profiles produced by selected enzymes, the *C. gigas* mitochondrial genome can be estimated at about 18.7 ± 0.2 kb. Heteroplasmy was observed in only one of the 159 animals sampled in British Columbia and Washington. This individual carried a size variant of approximately 50 bp detected in a *Hinc*II digest. There was little other evidence of size variation, although the methods of restriction fragment detection employed in this study would fail to detect size variants differing by 50 bp or less.

mtDNA Sequence Variation

The results indicate a very high level of haplotype diversity in these populations (Table 1). A total of 44 haplotypes were detected in the 141 animals from British Columbia. The smaller sample of 18 animals from Dabob, Washington, revealed an additional four haplotypes. Table 2 lists the approximate fragments sizes corresponding to the restriction profiles in Table 1. Two haplotypes (PS1 and PS2) were observed from all populations and one other (PS18) was found in low frequencies in the four British Columbia populations. Only two other haplotypes (PS3 and HT32) were observed in more than one population.

Each of the four British Columbia populations yielded 13 or 14 haplotypes in samples of 33–36 individuals (Table 1; Fig. 1). We would infer from these results that substantial genetic variation is present in these populations. Nucleon diversity indices (H; Nei and Tajima 1981) were calculated for each of the populations. For the British Columbia populations, H ranged from 0.78 (Hisnitt) to 0.82 (Pendrell) and differed very little in pairwise comparisons between populations. These values compare very closely with those obtained in a study of the American oyster, *Crassostrea virginica* (0.57 and 0.80 for the Atlantic and Gulf Coast populations, respectively; Reeb and Avise 1990). Only the Dabob Bay sample deviated substantially from the British Columbia values ($H = 0.57$). Since the Dabob sample is much smaller than the British Columbia samples, this deviation is not significant. These estimates imply that all populations have maintained high and relatively similar levels of genetic diversity.

Although we analysed only mtDNA sequence diversity, we believe that the result should extend to nuclear variation as well. Contemporary data regarding nuclear gene diversity in these populations are not available (however, see Buroker et al. 1975; Hedgecock and Sly 1990; Pogson 1991). Other studies (Smith et al. 1986; Chapman 1989; Hedgecock and Sly 1990; Sunden and Davis 1991) have shown that general levels of heterozygosity are maintained in recently bottlenecked populations but rare alleles are lost. The results reported here are in agreement with the high levels of nuclear allele diversity reported in these studies, but the precise relationship between rare mitochondrial haplotypes and rare nuclear alleles is not clear.

Is There Evidence of Geographic Subdivision?

The most striking feature of this data set lies in the frequency distributions of the observed haplotypes. Whereas about 60% of the oysters sampled fall into the two common haplotypes, almost 40% of individuals carried rare, often unique, variants (Table 1; Fig. 1). To determine whether this frequency profile could be explained by neutral theory, we performed a Ewens–Watterson test (Ewens 1972; Watterson 1978) comparing the observed value of $F = \sum p_i^2 = 0.188$ with values obtained in 2000 simulations of the test statistic (Stewart 1977; Manly 1985). In none of the simulations did the test statistic exceed the observed value of F, so we conclude that the haplotype distribution deviates significantly from neutral expectation ($p < 0.0005$). This deviation cannot be due to a systematic sampling error, since this study employed transects and a random number table to ensure random sampling of the populations. In addition, the fact that the same two haplotypes predominate in all British Columbia populations suggests that the results are an accurate reflection of the population haplotype distributions.

Frequency distributions with characteristics similar to those found here have been reported for the American oyster in eastern North America (Reeb and Avise 1990; Brown and Paynter 1991). Reeb and Avise found that most individuals of *C. virginica* fall into only two haplotypes, yet 80 rare ones were detected in the Gulf and Atlantic coast populations combined, in a total sample of 212 individuals. In their study, however, the two common haplotypes were geographically separated into Gulf Coast and Atlantic distributions and could be distinguished by restriction profiles produced by 8 of 13 restriction enzymes. A UPGMA tree of all 82 haplotypes showed them to be clearly divided into geographic groups (Reeb and Avise 1990).

In contrast, the two common haplotypes of Pacific oysters in British Columbia differed by only a single cut site in *Hind*III digests (Tables 1 and 2). A UPGMA tree of the 48 haplotypes observed in this study is given in Fig. 2. No clustering of genotypes with respect to geographic locale appears in this analysis. Similar results are obtained using neighbor-joining analysis. The tree is divided into two groups: those evidently derived from PS1 (top) and those derived from PS2 (bottom). As noted above, these two groups are separated by only a single cut site and perhaps only a single

TABLE 2. Fragment sizes for the restriction patterns observed for each of the polymorphic enzymes.

*Hpa*I
A: 7.50, 5.20, 1.85, 1.72, 1.28, 0.51
B: 7.50, 5.20, 2.35, 1.72, 1.28
C: 7.50, 3.00, 2.20, 1.85, 1.72, 1.28, 0.51

*Hin*dIII
A: 4.10, 3.30, 2.90, 2.10, 2.10, 1.70, 0.84, 0.60, 0.48, 0.38, 0.38, 0.31
B: 4.10, 3.30, 2.90, 2.10, 2.10, 2.05, 0.84, 0.60, 0.48, 0.38, 0.31
C: 4.10, 3.30, 2.90, 2.10, 2.10, 2.05, 0.88, 0.60, 0.48, 0.38, 0.31
D: 4.10, 2.90, 2.10, 2.10, 2.05, 2.05, 1.20, 0.84, 0.60, 0.48, 0.38, 0.31
E: 3.30, 2.90, 2.10, 2.10, 2.10, 2.00, 1.70, 0.84, 0.60, 0.48, 0.38, 0.38, 0.31
F: 4.10, 2.90, 2.40, 2.10, 2.10, 2.05, 0.84, 0.78, 0.60, 0.48, 0.38, 0.31
G: 4.10, 3.30, 2.90, 2.40, 2.10, 2.05, 0.84, 0.60, 0.48, 0.31
H: 4.10, 3.30, 2.90, 2.10, 2.05, 1.80, 0.84, 0.60, 0.48, 0.38, 0.31, 0.31

*Hae*III
A: 2.60, 1.90, 1.35, 1.35, 1.28, 1.13, 1.00, 0.94, 0.94, 0.86, 0.84, 0.74, 0.74, 0.54, 0.50, 0.50, 0.44, 0.39, 0.33, 0.26, 0.23, 0.17
B: 1.95, 1.90, 1.35, 1.35, 1.28, 1.00, 0.94, 0.94, 0.86, 0.84, 0.74, 0.74, 0.62, 0.54, 0.50, 0.50, 0.44, 0.39, 0.33, 0.26, 0.23, 0.17
C: 2.60, 1.90, 1,35, 1.35, 1.28, 1.13, 1.00, 0.94, 0.94, 0.86, 0.84, 0.74, 0.67, 0.54, 0.50, 0.50, 0.44, 0.39, 0.33, 0.26, 0.23, 0.17
D: 2.60, 1.90, 1.35, 1.35, 1.32, 1.28, 1.00, 0.94, 0.94, 0.86, 0.84, 0.74, 0.74, 0.54, 0.50, 0.50, 0.44, 0.39, 0.33, 0.26, 0.23
E: 2.60, 1.90, 1.35, 1.35, 1.28, 1.00, 0.94, 0.94, 0.86, 0.84, 0.74, 0.74, 0.54, 0.54, 0.50, 0.50, 0.44, 0.39, 0.33, 0.26, 0.23, 0.17
F: 2.60, 1.90, 1.35, 1.35, 1.28, 1.00, 1.00, 0.94, 0.94, 0.86, 0.84, 0.74, 0.74, 0.54, 0.50, 0.50, 0.44, 0.39, 0.33, 0.26, 0.23, 0.17
G: 2.60, 1.90, 1.35, 1.35, 1.13, 1.00, 0.94, 0.94, 0.86, 0.84, 0.74, 0.74, 0.74, 0.54, 0.50, 0.50, 0.50, 0.44, 0.39, 0.33, 0.26, 0.23, 0.17
H: 2.60, 1.90, 1.35, 1.35, 1.30, 1.28, 1.00, 0.94, 0.94, 0.86, 0.84, 0.74, 0.74, 0.54, 0.50, 0.50, 0.44, 0.39, 0.33, 0.26, 0.23
I: 2.60, 1.90, 1.35, 1.35, 1.28; 1.28, 1.00, 0.94, 0.94, 0.86, 0.84, 0.74, 0.74, 0.54, 0.50, 0.50, 0.44, 0.39, 0.33, 0.26, 0.23, 0.17
J: 2.60, 1.90, 1.35, 1.35, 1.28, 1.13, 1.00, 0.94, 0.94, 0.86, 0.84, 0.74, 0.74, 0.54, 0.50, 0.50, 0.44, 0.39, 0.39, 0.33, 0.26
K: 2.60, 1.90, 1.35, 1.35, 1.28, 1.13, 1.00, 0.94, 0.94, 0.86, 0.84, 0.74, 0.74, 0.50, 0.50, 0.44, 0.39, 0.33, 0.26, 0.23, 0.17; 0.17
L: 2.60, 1.90, 1.35, 1.35, 1.28, 1.13, 1.00, 0.94, 0.94, 0.86, 0.84, 0.74, 0.74, 0.60, 0.54, 0.50, 0.50, 0.44, 0.39, 0.23, 0.17
M: 2.60, 1.90, 1.35, 1.35, 1.28, 1.13, 0.94, 0.94, 0.90; 0.86, 0.84, 0.74, 0.74, 0.54, 0.50, 0.50, 0.44, 0.39, 0.26, 0.23, 0.17
N: 2.60, 2.30, 1.90, 1.35, 1.35, 1.13, 0.94, 0.94, 0.86, 0.84, 0.74, 0.74, 0.54, 0.50, 0.50, 0.44, 0.39, 0.33, 0.26, 0.23, 0.17

*Hpa*II
A: 3.70, 3.55, 3.10, 2.20, 1.48, 1.36, 0.90, 0.67, 0.59, 0.58, 0.52, 0.28
B: 3.70, 3.55, 3.10, 2.20, 1.48, 1,36, 1.10, 0.90, 0.67, 0.59, 0.28
C: 3.70, 3.10, 2.55, 2.20, 1.48, 1.36, 0.98, 0.90, 0.67, 0.59, 0.58, 0.52, 0.28
D: 3.55, 3.10, 2.20, 2.10, 1.60, 1.48, 1.36, 0.90, 0.67, 0.59, 0.58, 0.52, 0.28
E: 3.70, 3.55, 3.10, 2.20, 1.48, 1.00, 0.90, 0.67, 0.59, 0.58, 0.52, 0.40, 0.28
F: 3.70, 3.10, 2.20, 2.10, 1.48, 1.40, 1.36, 0.90, 0.67, 0.59, 0.58, 0.52, 0.28
G: 3.70, 3.55, 3.10, 2.20, 1.48, 1.36, 0.90, 0.67, 0.58, 0.52, 0.52, 0.28
H: 3.70, 3.10, 2.35, 2.20, 1.48, 1.36, 1.22, 0.90, 0.67, 0.59, 0.58, 0.52, 0.28
I: 3.70, 3.55, 3.10, 2.20, 1.93, 1.36, 0.90, 0.67, 0.59, 0.58, 0.28
J: 3.70, 3.55, 3.10, 2.20, 1.48, 1.36, 0.90, 0.67, 0.59, 0.58, 0.52, 0.32

*Ava*I
A: 9.80, 4.20, 2.90, 2.25, 0.52
B: 10.30, 4.20, 2.90, 2.25

*Hae*II
A: 5.05, 4.70, 3.30, 2.33, 2.15, 1.70, 0.38
B: 5.40, 5.05, 4.70, 2.33, 1.70, 0.38
C: 5.05, 4.70, 2.33, 2.15, 1.70, 1.65, 1.45, 0.38
D: 5.05, 5.00, 4.70, 2.33, 2.15, 0.38
E: 5.05, 3.30, 3.15, 2.33, 2.15, 1.70, 1.60, 0.38

nucleotide substitution. They clearly do not represent the extent of subdivision observed in the American oyster (Reeb and Avise 1990), in terms of either sequence divergence or geographic separation.

The species was only recently introduced from Japan and has successfully reproduced only occasionally in British Columbia (Numachi 1978; Quayle 1988). Genetic differences might have been established in the absence of gene

TABLE 2. (*Concluded*)

*Hinc*II
A: 3.25, 2.46, 2.34, 1.92, 1.82, 1.70, 1.22, 1.10, 0.86, 0.60, 0.60, 0.46
B: 3.25, 2.46, 2.34, 2.28, 1.92, 1.70, 1.22, 1.10, 0.86, 0.60, 0.60, 0.46
C: 3.25, 2.46, 2.34, 1.92, 1.76, 1.70, 1.22, 1.10, 0.86, 0.60, 0.60, 0.46

*Ava*II
A: 3.20, 2.75, 2.45, 2.05, 2.05, 1.75, 1.30, 0.83, 0.78, 0.77
B: 4.50, 3.20, 2.45, 2.05, 2.05, 1.30, 0.83, 0.78, 0.77
C: 3.20, 2.75, 2.05, 2.05, 1.75, 1.30, 1.30, 1.20, 0.83, 0.78, 0.77
D: 3.20, 2.45, 2.05, 2.05, 1.75, 1.45, 1.30, 1.30, 0.83, 0.78, 0.77
E: 4.50, 3.20, 2.75, 2.05, 1.75, 1.30, 0.83, 0.78, 0.77
F: 3.20, 2.75, 2.25, 2.05, 2.05, 1.75, 1.30, 0.83, 0.78, 0.77, 0.20

*Hin*fI
A: 1.53, 1.40, 1.24, 1.22, 1.13, 1.02, 1.02, 0.91, 0.90, 0.76, 0.68, 0.68, 0.68, 0.62, 0.56, 0.56, 0.48, 0.36, 0.17
B: 1.53, 1.40, 1.24, 1.22, 1.13, 1.02, 1.02, 0.91, 0.90, 0.85; 0.76, 0.68, 0.68, 0.68, 0.62, 0.56, 0.56, 0.17
C: 1.53, 1.40, 1.24, 1.22, 1.13, 1.02, 1.02, 0.91, 0.90, 0.76, 0.68, 0.68, 0.68, 0.62, 0.56, 0.56, 0.48, 0.36
D: 1.53, 1.40, 1.24, 1.13, 1.02, 1.02, 0.98, 0.91, 0.90, 0.76, 0.68, 0.68, 0.68, 0.62, 0.56, 0.56, 0.48, 0.36, 0.26, 0.17
E: 1.53, 1.40, 1.24, 1.22, 1.13, 1.02, 1.02, 0.91, 0.90, 0.76, 0.70, 0.68, 0.68, 0.68, 0.62, 0.56, 0.56, 0.48, 0.40, 0.36, 0.17
F: 1.90, 1.53, 1.40, 1.24, 1.13, 1.02, 1.02, 0.91, 0.90, 0.76, 0.68, 0.68, 0.62, 0.56, 0.56, 0.48, 0.36, 0.17
G: 1.53, 1.40, 1.24, 1.22, 1.13, 1.02, 1.02, 0.91, 0.90, 0.76, 0.68, 0.68, 0.68, 0.62, 0.56, 0.56, 0.36, 0.17
H: 1.53, 1.40, 1.24, 1.22, 1.02, 1.02, 0.91, 0.90, 0.85, 0.76, 0.68, 0.68, 0.68, 0.62, 0.56, 0.56, 0.48, 0.36, 0.17, 0.17
I: 1.53, 1.40, 1.22, 1.17, 1.13, 1.02, 1.02, 0.91, 0.90, 0.76, 0.68, 0.68, 0.68, 0.62, 0.56, 0.56, 0.48, 0.36, 0.17

TABLE 3. Mean percent divergence estimates among populations (below the diagonal) and nucleotide diversity estimates (above) among *C. gigas* populations. The average percent nucleotide divergence and average nucleotide diversity for all populations are 0.011 and 0.159, respectively.

	Pipestem	Pendrell	Hotham	Hisnitt	Dabob
Pipestem		0.160	0.187	0.159	0.158
Pendrell	0.002		0.176	0.158	0.146
Hotham	0.028	0.013		0.170	0.132
Hisnitt	0.003	<0.002	0.010		0.139
Dabob	0.030	0.015	<0.002	0.011	

flow, either at the time of introduction or as a result of local differences in reproduction or survival of lineages. As noted above, the two common haplotypes, PS1 and PS2, are the predominant variants in all British Columbia populations, but each population has about 10 haplotypes not observed in any other population (Table 1; Fig. 1). The existence of "private" alleles has been used as evidence of geographic differentiation (Slatkin 1985). It should be noted, however, that of the 39 private haplotypes, only five were encountered more than once in the population of their origin. That is, 34 were not encountered again in any population, including the one where it was observed. We believe that the sample sizes in this study are insufficient to demonstrate that these haplotypes are truly private.

The above analysis treats haplotypes as alleles, without regard to the number of nucleotide substitutions distinguishing them. Nucleotide divergence between haplotypes can be calculated for restriction analysis studies and can be exploited as an additional test for population divergence. Table 3 gives a pairwise comparison of nucleotide divergence between populations (below the diagonal) with the total nucleotide diversity in each pair of populations (above the diagonal). Nucleotide divergence represents the proportion of the nucleotide diversity, in that population pair, that cannot be explained by the within-population diversity. On average, only about 7% of the nucleotide diversity is due to divergence between populations.

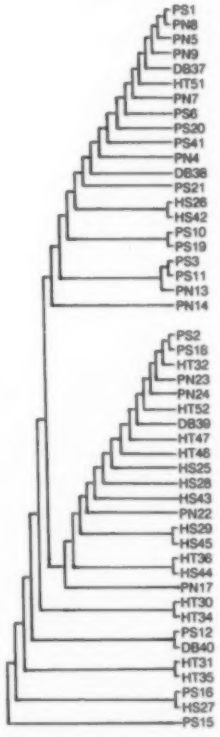

FIG. 2. UPGMA dendrogram generated from sequence divergence estimates for all haplotypes. The dendrogram was generated using Phylip version 3.4 (Felsenstein 1991).

The British Columbia populations of *C. gigas* have been established for less than 60 yr. Considerable expanses of

cold ocean waters present geographic obstacles to larval survival and dispersal between the separated west coast populations (Hisnitt and Pipestem) and between these and the Strait of Georgia populations (Quayle 1988). The Strait of Georgia populations were established by natural dispersal mechanisms, but we would expect the amount of gene flow to be greatly restricted. Although a majority of haplotypes are not shared among the introduced populations, the common haplotypes are shared at comparable frequencies. This finding contrasts sharply with the observations on *C. virginica* (Reeb and Avise 1990). Not only are the common haplotypes not shared, they and the associated rare haplotypes differ at a large number of sites. Insufficient time has passed since the introduction of *C. gigas* to North America for genetic divergence to have become established.

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Effect of Current Velocity on Ingestion Rates of Black Fly Larvae

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In situ feeding measurements were made to quantify the effect of current velocity on ingestion rates of larvae of the major pest species of black flies in southern Quebec (*Simulium venustum/verecundum*, *S. tuberosum*, and *Prosimulium mixtum/fuscum*) and to compare these results with those obtained in seminatural conditions (gutter system). Ingestion rates of all three species increased with current velocity by a factor of 2.5 (*P. mixtum/fuscum*) to 10 (*S. tuberosum*) between 25 and 100 $cm \cdot s^{-1}$. Measured field rates were significantly correlated with those predicted for similar larval sizes, temperatures, and seston concentrations in gutters, although field rates were only 56–87% of those in gutters once corrected for differences in current velocity. These results suggest that the preference of these species for current velocities of about 100 $cm \cdot s^{-1}$ reflect their ability to feed faster at these velocities. The effectiveness of larvicides can therefore be strongly affected by current velocities, and determinations of effective doses from gutter experiments may slightly underestimate the required quantity of larvicide.

L'alimentation in situ a été mesurée afin de quantifier l'effet de la vitesse du courant sur les taux d'ingestion des larves des principales espèces nuisibles de mouche noire dans le sud du Québec (*Simulium venustum/verecundum*, *S. tuberosum* et *Prosimulium mixtum/fuscum*) ainsi que pour comparer ces résultats à ceux obtenus dans des conditions semi-naturelles (système à gouttières). Les taux d'ingestion des trois espèces augmentent avec la vitesse du courant d'un facteur de 2,5 (*P. mixtum/fuscum*) à 10 (*S. tuberosum*) entre 25 et 100 $cm \cdot s^{-1}$. On a observé une corrélation significative entre les taux mesurés dans des conditions naturelles et ceux prédits pour des conditions similaires en ce qui concerne la taille des larves, la température et la concentration du seston dans les gouttières; cependant, les taux naturels ne correspondent qu'à 56 à 87 % de ceux mesurés dans les gouttières, une fois la correction effectuée pour tenir compte des différences de vitesse du courant. Ces résultats semblent indiquer que la préférence de ces espèces pour une vitesse de courant d'environ 100 $cm \cdot s^{-1}$ reflète leur capacité de s'alimenter plus rapidement dans ces conditions. Par conséquent, l'efficacité des larvicides peut être fortement altérée par la vitesse du courant, et la détermination des doses efficaces à partir d'expériences menées en gouttières pourraient légèrement sous-estimer la quantité de larvicide requise.

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Larvae of black flies (Diptera: Simuliidae) are common inhabitants of flowing water systems. Attached to hard substrates, they feed by filtering particulate matter in flowing waters with specialized labral fans (Chance and Craig 1986). They often attain high densities and biomass in lake outlets where their growth depends mainly on the bacterio- and phytoplankton exported from the lake (Wotton 1979, 1982; Gislason 1985). In these systems, biomass is sometimes sufficiently high that black fly populations can effectively ingest most of the small particles exported from a lake within less than 100 m from the outfall (Morin et al. 1988a). These populations therefore have the potential to alter the flux of energy down the stream continuum by sequestering nutrients and potentially pollutants. Estimation of the impact of these populations on transport of particles, energy, and pollutants requires measurements of their feeding rates under natural conditions. In addition, because most control agents of larval black flies must be ingested to be effective (Lacey and Undeen 1986; Merritt et al. 1989; Morin et al. 1989; Atwood et al. 1992), there are also immediately practical reasons to estimate feeding rates in the field, since they determine the efficacy of these larvicides.

Morin et al. (1988b) combined the results of several feeding experiments conducted under seminatural conditions (a gutter system fed with stream water) in a multiple regression model that describes how larval body size, water temperature, and the concentration of suspended particles (seston) affect larval ingestion rates. The model was then combined with results of bioassays performed under similar conditions to establish the required dose of *Bacillus thuringiensis israelensis* (*B.t.i.*) for controlling black fly populations (Morin et al. 1989). These calculations did not include the effects of current velocity on larval ingestion rates. The assumption was also made that ingestion rates in the field were similar to those observed using the gutter system.

Although black fly larvae depend on the current to capture their food, the literature provides conflicting views on the effects of current velocity on larval ingestion rates. Some studies suggest that ingestion rates increase with increasing velocity (Schröder 1980), others that they decrease (Ciborowski and Craig 1989), and still others that they remain constant (Thompson 1987). This apparent contra-

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diction may be explained by the range of velocities studied if the relationship between ingestion rate and current is curvilinear, peaking at intermediate velocities (Lacey and Mulla 1979). Because filtration is largely passive, one might expect that at low current velocities, ingestion rates would increase with an increased flux of particles generated by higher velocities. However, rates must eventually plateau when the ingestion rate becomes limited by handling time (Hart and Latta 1986) and should decrease at very high velocities when drag starts to interfere with feeding (Chance and Craig 1986; Lacoursière 1992).

Existing data on ingestion rates at various velocities cannot easily be combined to yield a general pattern because of differences in the spatial scale at which velocity measurements were made and because of differences in other hydrodynamic properties (e.g., depth, bed roughness, density of conspecifics). Further, due to friction, velocity is lower next to the body of a larva attached to hard substrate than in the water column above it (Vogel 1981). Depending on the method of measurement or the size of the recording device, measurements of velocity will also vary. For example, velocities measured in a streambed with standard devices (e.g., Pygmy current meters) cannot easily be compared with velocities measured in experimental flumes or those calculated from the movement of small particles past the filtering apparatus of larvae.

In this study, we quantify the effect of current velocity, as measured with a Pygmy current meter, on ingestion rates of the major pest species of simuliid larvae in southern Quebec. We then test published models of larval ingestion rates (Morin et al. 1988b) by comparing ingestion rates observed in streams with those predicted by the model.

Materials and Methods

The study was conducted during the third week of May 1989 and on March 4 and 5, 1990. The May experiments, involving *Simulium venustum/verecundum* and *S. tuberosum*, were conducted in Ruisseau Fraser located at the Réserve Saint-Maurice, Quebec (46°57′N, 73°02′W). The March study on *Prosimulium mixtum/fuscum* was performed at the outlets of Lac Bleu, Lac Croche, and Lac Pin Rouge in the lower Laurentians of Quebec (46°00′N, 74°00′W). In each stream the experiments were conducted at current velocities ranging from 24 to 133 cm·sec^{-1} at a depth of approximately 6 cm.

Ingestion experiments were conducted where high densities of larvae were attached to easily removed substrates such as branches or rocks. Six or seven experimental sites, within a 20-m stream reach, were used in each stream to sample as wide a range of velocities as possible. Prior to each experiment, we recorded water temperatures and measured current velocities at the site by holding a Gurley Pygmy current meter 2 cm above the larvae. Triplicate water samples (4.5 L each) were also collected in each stream to determine seston concentrations. The water was later filtered (500–2000 mL, depending on concentration of particles) through preweighed GF/C glass microfiber filters that were left to dry in a desiccator for 24 h before being reweighed.

The protocol for the ingestion experiments was the same for all studies and is similar to that of Morin et al. (1988b). Each ingestion experiment started when a suspension of powdered charcoal (10 g in 500 mL of stream water) was released into the water immediately upstream of the designated site for 2–3 min. During this interval the larvae ingested some of the powdered charcoal. Following exposure to charcoal, the larvae were left to feed on natural seston. During the May experiments (water temperatures 13–14°C) the larvae were allowed to feed on seston for 10–15 min. However, during the March experiments when the temperature did not exceed 1°C and ingestion rates were lower, the larvae were left to feed on natural seston for periods of 60–120 min. To mark the end of the experiment the larvae were once again exposed to the charcoal suspension for 2–3 min. Five minutes after the second application of powdered charcoal, the larvae were collected and preserved in a 4% formaldehyde solution. Because the particles ingested by black fly larvae are not mixed during passage through the cylindrical gut, the charcoal ingested at the beginning and at the end of the experiment formed two distinctive black bands clearly visible under a dissecting microscope.

All larvae were identified. For each specimen, body length (L, millimetres) and the length and width of the plug between the two charcoal bands were recorded. These measurements were used to calculate larval ingestion rates expressed as the volume of gut filled per unit time.

Data Analyses

To quantify the effect of current velocity on ingestion rates of the larvae, we first adjusted observed ingestion rates for differences in size by calculating the residuals (observed values minus predicted) to the model

$$(1)\quad \log_{10} I = a + \log_{10} L$$

where I is ingestion rate (cubic millimetres per minute) and L is body length (millimetres). Separate regressions were fitted for each species. Average residual values for each current speed were regressed against velocity (centimetres per second) using weighted polynomial regression to account for differences in sample size at each velocity (weight inversely proportional to the variance of the mean residual).

To compare ingestion rates under natural conditions with those measured previously in the gutter system, we regressed the measured ingestion rates on natural substrate on those predicted by the equation developed by Morin et al. (1988b) for *S. venustum/verecundum* larvae feeding in gutters at current velocities averaging 50 cm·s^{-1}:

$$(2)\quad \log_{10} I = -4.89 + 2.4\log_{10} L + 0.037\,T - \frac{0.58}{S} + 0.6\,\frac{\log_{10} L}{S}$$

where T is water temperature (degrees Celcius) and S is seston concentration (milligrams per litre).

Results and Discussion

Effect of Body Length and Current Velocity

Ingestion rates increased with increasing body length and current velocity for larvae of the three species (Table 1; Fig. 1). Ingestion rates scaled roughly as the cube of length for the three species, and body length accounted for 49, 40, and 61% of the variance in log-transformed ingestion rates for *S. venustum/verecundum*, *S. tuberosum*, and *P. mixtum/fuscum*, respectively. Although ingestion rates were affected by velocity in all species studied, the effect of velocity varied among the three species. For all species, inges-

TABLE 1. Coefficients (±1 SE) and statistics of the multiple regression models relating $\log_{10}$ ingestion rates (log I, $mm^3 \cdot mm^{-1}$) to $\log_{10}$ body length (log L, mm). All listed coefficients are highly significant ($P < 0.0001$). Experiments with *S. venustum/verecundum* and *S. tuberosum* were performed at 13°C and seston concentrations of 2.2 mg dry matter·L^{-1}. Those with *P. mixtum/fuscum* were performed at 1°C in water containing 0.4 mg dry matter·L seston^{-1}.

Parameter	*S. venustum/verecundum*	*S. tuberosum*	*P. mixtum/fuscum*
Constant	−5.34±0.08	−5.12±0.27	−6.17±0.06
log L	3.41±0.12	3.99±0.45	3.37±0.08
Residual variance	0.18	0.15	0.06
R^2	0.49	0.40	0.61
n	776	118	1022

tion rates were maximum at the highest velocities tested, although ingestion rates peaked significantly ($P < 0.01$ for the quadratic term in Eq. 1) within the range of velocities tested only for *S. tuberosum*. Moreover, the intensity of the ingestion response to changes in current velocity varied among species. From 25 to 100 cm·s^{-1}, ingestion rates of *S. venustum* increased about fourfold, those of *S. tuberosum* about 10-fold, and those of *P. mixtum/fuscum* by about 2.5-fold (Fig. 1). Although velocity explained most of the variability among mean residuals, it accounted for substantially less of the variance in log-transformed ingestion rates measured for individual larvae than body length for *S. venustum/verecundum* (7%) and *P. mixtum/fuscum* (5%) but not for *S. tuberosum* (39%).

Ingestion rates of black fly larvae are not a simple function of current velocity. The flux (particle concentration times velocity) increases with velocity, but the ability of feeding appendages to intercept the particles decreases at higher velocities (Hart et al. 1991) and eventually the rate at which particles are transferred to the gut stabilizes. Under natural stream conditions, we found that ingestion rates were highest at highest velocities where larvae were found at high densities. Similar observations were made by Hansen et al. (1991) in a study where velocity preferences of black fly larvae were determined by exposing larvae to a gradient of current velocities in a controlled flow chamber. The biomass of black fly larvae increases in response to increased current velocity up to about 100–120 cm·s^{-1} and then starts to decline (Morin and Peters 1988; Morin 1991). The negative effect of fast water currents on the abundance of *S. tuberosum* larvae may be related to reduced ingestion rates, perhaps caused by the deformation of labral fans due to high drag forces of the water (Craig and Chance 1982; Lacoursière 1992). The same may apply to the two other species groups (*S. venustum/verecundum* and *P. mixtum/fuscum*), although feeding experiments in faster currents would be required to test this speculation.

Differences in current velocities translated into approximately proportional changes in ingestion rates for *S. venustum/verecundum* and *P. mixtum/fuscum*, but the 10-fold increase in ingestion rate of *S. tuberosum* larvae when velocity rises from 25 to 100 cm·s^{-1} exceeded the estimated fourfold increase in particle flux. Morin et al. (1988b) determined that, on average, *S. tuberosum* larvae ingest 150% of the volume ingested per unit time by *S. venustum* larvae of similar sizes and under the same conditions. The morphological differences that underlie these differences in efficiency of particle capture may also be responsible for their different responses to increased velocities.

Test of the Predictive Model

Observed field ingestion rates correlated significantly with rates predicted for *S. venustum/verecundum* in seminatural conditions by the model of Morin et al. (1988b), although larvae appeared to feed less in natural conditions than in the gutter system of Morin et al. (1988b) once ingestion rates were corrected for size, temperature, and seston concentration. Direct comparison of observed rates in the stream with those predicted in gutters (Fig. 2) suggested that *S. venustum/verecundum* fed in the stream at only 59% of the rate predicted for gutters, *S. tuberosum* fed at 204% the rate of *S. venustum/verecundum* in gutters, and *P. mixtum/fuscum* fed at 85% of the predicted rate. Direct comparisons are biased, however, by the differences in current velocities among experiments because Morin et al. (1988b) measured rates only at 50 cm·s^{-1}. Once corrected for current velocity using the regressions of Fig. 1, ingestion rates of all three species were found to be lower in streams than those predicted for gutters (field rates average 56, 64, and 87% of gutter rates for *S. venustum/verecundum*, *S. tuberosum*, and *P. mixtum/fuscum*, respectively). The most accurate predictions were made for the ingestion rate of *P. mixtum/fuscum*, despite the fact that these predictions are for conditions well outside the range of seston concentrations and temperatures originally considered.

Several factors may explain differences between ingestion rates in natural and seminatural conditions, although we suspect that gutter experiments do provide good approximations of natural feeding rates. Differences may result from higher flow heterogeneity and turbulence in stream conditions, methodological differences in flow measurement, or differences in the particles available to larvae. The flow conditions maintained in the gutter system over a smooth surface were certainly more homogeneous than what is experienced by larvae in natural streams. This greater heterogeneity in flow conditions probably contributed to the higher variability in ingestion rates in the stream compared with those in gutter systems (residual variances for models of Table 1 are higher than most of those reported by Morin et al. (1988b) for gutter experiments) and may have decreased average ingestion rates. Measurements of velocity in gutters were made by observing the rate of displacement of a plug of dye. This method allows measurement of the average velocity of the water column which is usually higher than what is measured 2 cm over the substrate by a Pygmy current meter (personal observation). Therefore, the velocity in gutters was probably overestimated compared with what was measured in the stream, which

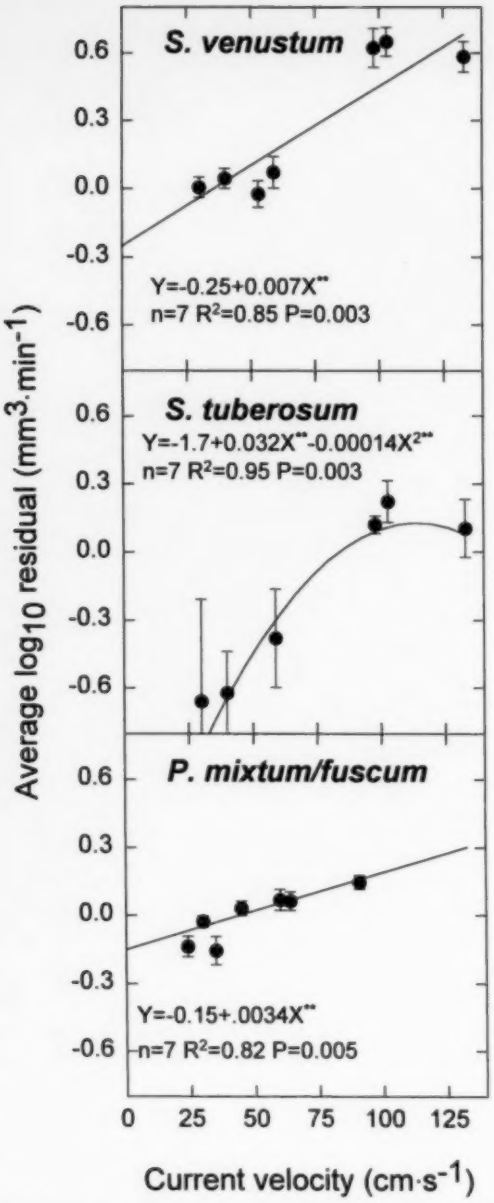

FIG. 1. Effect of velocity on ingestion rates of *S. venustum*, *S. tuberosum*, and *P. mixtum/fuscum* larvae. Each point represents the average ingestion rate of larvae for one experiment corrected for body size. Vertical bars represent 95% confidence intervals for the mean, and the lines are the weighted regressions.

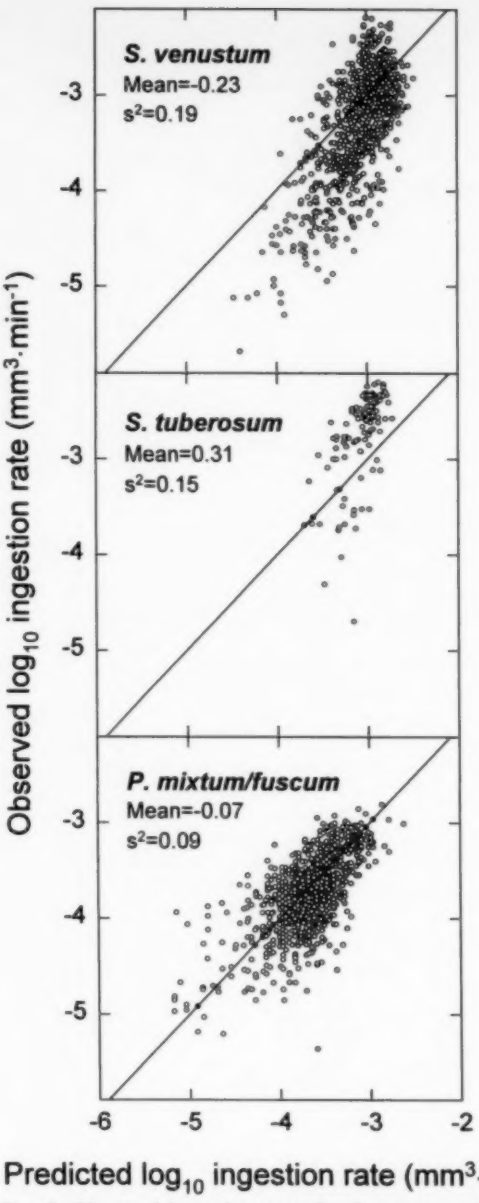

FIG. 2. Observed ingestion rates for *S. venustum* (n = 776), *S. tuberosum* (n = 118), and *P. mixtum/fuscum* (n = 1022) versus ingestion rates predicted by the model of Morin et al. (1988b) for *S. venustum/verecundum* larvae at 50 cm·s^{-1}. The diagonal line is the 1:1 line. The mean residual to the published model (observed minus predicted) is listed with its variance (s^2).

would tend to make field values less than gutter values. Finally, the particles available to larvae in gutter experiments were probably of a different nature than those ingested by stream larvae because of the differences in season and location. The similarity in seston concentrations may in fact hide a dissimilarity in particle size that affects ingestion rates (Kurtak 1978; Braimah 1987). Considering all these uncontrolled sources of variations, gutter experiments provide good, although slightly biased, estimates of feeding rates in natural conditions.

Relative Importance of Current Velocity on Feeding Rates

The relative importance of velocity as a determinant of ingestion rates can be determined by examining the predicted ingestion rates (Morin et al. 1988b) for larvae of different

sizes, for different temperatures and different seston concentrations, and comparing those obtained when current velocity is taken into consideration. According to the models of Morin et al. (1988b) and the results of this study, body size strongly influences ingestion rate: 10-mm larvae ingest about 125 times more particles per unit time than do 2-mm larvae, although per unit mass, their ingestion rates are of the same order of magnitude. A 10°C change in water temperature causes larval ingestion rates to change by a factor of 2. Similarly, changes in seston concentration from 2 to 10 $mg \cdot L^{-1}$ cause an increase in ingestion rates of about 50%. On the other hand, changes in current velocity from 25 to 100 $cm \cdot s^{-1}$ (within the tolerance range of most species) result in ingestion rates increasing 2.5- to 10-fold. Therefore, current velocity may well be more important than body length, seston, or temperature in determining larval feeding rates. This strong influence of current velocity on feeding may explain the higher densities of larvae observed in fast current when current velocities are measured at a similar scale (Morin and Peters 1988).

Practical Implications

Because current velocity affects feeding rates of larvae, it will influence significantly the efficacy of herbicides. In practice, because current velocity is rarely controlled in streams and rivers, it is mainly when screening larvicide formulations and estimating field mortality rates after a larvicide application (Walsh 1985; Barton et al. 1991) that current velocity should be taken into account. Velocities have to be standardized for proper comparisons of larvicidal effectiveness or to properly describe their downstream carry. Because ingestion rates increase with velocity, calculation of mortality made at high velocities may overestimate real lethality for a given dosage. Effective control may be obtained by calculating the required doses for larvae in slow currents; such doses would ensure sufficient mortality in faster currents.

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Downstream Movement and Mortality of Brook Trout (*Salvelinus fontinalis*) Exposed to Acidic Episodes in Streams

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Episodes of low pH and high Al concentration were associated with net downstream movement and increased mortality of ratio-tagged brook trout (*Salvelinus fontinalis*). We compared movements of 10–15 brook trout in two streams with severe acidic episodes with movements of fish in two streams with less severe acidic episodes (reference streams). Study populations moved downstream hundreds of metres in the streams with more severe acidic episodes (pH <5.0 and >200 μg Al/L). Median downstream movement in spring was 250 and 900 m after 20 d for fish in the more acidic streams; one third of the fish were found dead during this time. We found no net movement and no dead fish in the reference streams. Lower stream discharge in fall studies was associated with less severe acidic episodes, less net movement, and no mortality. Water samples collected at individual fish locations indicated that few fish avoided adverse effects of acidic episodes by remaining in microhabitats with higher pH and lower Al concentration.

Les épisodes de faible pH et de concentration élevée d'Al ont été associés à un mouvement net vers l'aval et à une augmentation de la mortalité de l'omble de fontaine (*Salvelinus fontinalis*), grâce à des expériences de radio-marquage. Nous avons comparé les mouvements de 10 à 15 ombles de fontaine dans deux cours d'eau où se produisent des épisodes acides aigus à ceux des poissons dans deux cours d'eau connaissant des épisodes acides moins importants (cours d'eau de référence). Dans les cours d'eau connaissant des épisodes acides plus aigus (pH <5,0 et >200 μg Al/L), les populations étudiées se sont déplacées de plusieurs centaines de mètres vers l'aval. Le mouvement vers l'aval médian au printemps était de 250 et 900 m après 20 jours pour les poissons dans les cours d'eau plus acides; un tiers des poissons ont été retrouvés morts au cours de cette période. Nous n'avons observé aucun mouvement net et aucune mortalité de poisson dans les cours d'eau de référence. Dans les études réalisées à l'automne, un débit plus faible des cours d'eau a été associé à des épisodes acides moins aigus, un mouvement net moindre et aucune mortalité. Les échantillons d'eau prélevés aux emplacements où vivaient les poissons ont indiqué que peu de poissons évitaient les effets nocifs des épisodes acides en demeurant dans des microhabitats présentant un pH plus élevé et une concentration d'Al plus faible.

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Atmospheric deposition has been linked to short-term decreases in pH and increases in Al concentration during high stream discharge (Sharpe et al. 1984). These acidic episodes have been widely documented in poorly buffered streams (Reader and Dempsey 1989; Wigington et al. 1990). Assessing effects of acidic deposition on fish requires knowledge of how fish respond to the rapid temporal variation in water chemistry. Furthermore, within an acidic stream network, spring seeps and tributaries can potentially act as more alkaline microhabitats of higher pH and lower Al concentration (e.g., Driscoll et al. 1987; Sharpe and DeWalle 1990).

Downstream movement of salmonids has been observed during acidic episodes (Leivestad and Muniz 1976; Hall et al. 1980; Skogheim et al. 1984; Gagen et al. 1989) and several field studies have reported apparent congregations of fish near the inflow points of more alkaline tributaries

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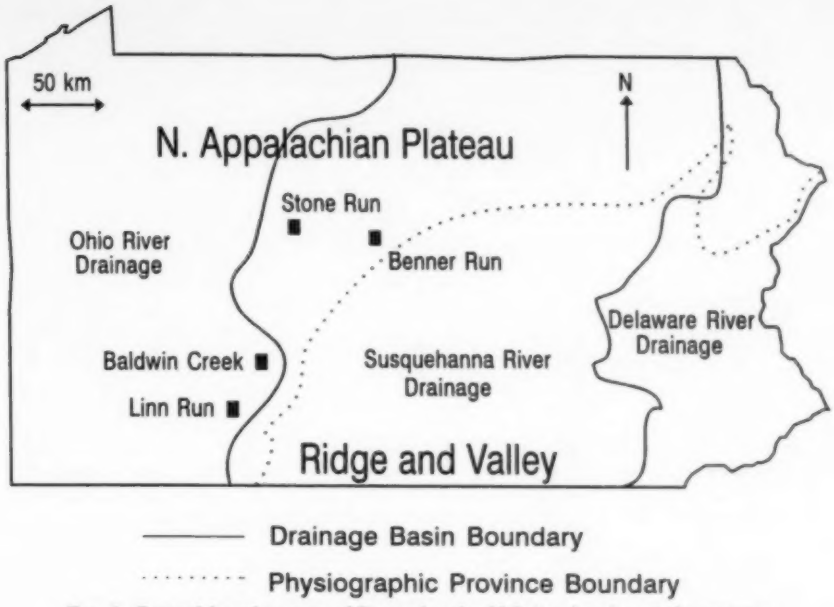

FIG. 1. General location map of Pennsylvania (U.S.A.) showing study streams.

(Muniz and Leivestad 1981; Watt et al. 1983). Gloss et al. (1989) documented mass movement of hatchery-reared brook trout (*Salvelinus fontinalis*) from a recently neutralized lake that was undergoing reacidification. Numbers of fish leaving another neutralized lake, which did not reacidify, remained stable. None of these field studies was designed specifically to measure rate of movement or overall distance traveled or to track changes in the water chemistry.

Several laboratory studies have shown that, when given an opportunity, fish avoid water of low pH and high Al concentration (Johnson and Webster 1977; Jones et al. 1985; Gunn and Noakes 1986; Nakamura 1986). Breck et al. (1988) used computer simulation to demonstrate that behavioral avoidance of episodes could greatly increase the probability of fish populations surviving acidic episodes, yet the observation by Gunn and Noakes in 1986 (p. 502) that "...to date, there are no detailed *in-situ* studies of avoidance reactions to acidic water for any fish species" is still valid.

This study was designed to determine whether acidic episodes affect direction (upstream versus downstream) and distance of movements by brook trout in headwater streams and if fish moved to areas of lower Al concentration. Brook trout were selected as a test organism because (1) they are widely distributed in streams vulnerable to acidic episodes, (2) many studies have documented physiological responses of brook trout to low pH and high Al concentration, (3) their avoidance behavior has been described in laboratory studies, and (4) their high mobility seemed to make them a likely species that would use alkaline microhabitats during acidic episodes.

Materials and Methods

Miniature radio transmitters (1.5–2.0 × 0.75 × 0.90 cm, weight 1.7–2.5 g) were placed in brook trout that were then released in a pair of streams differing in severity of acidic episodes. One pair of streams, Linn Run and Baldwin Creek, were 30 km apart and the other pair, Stone Run and Benner Run, were 40 km apart (Fig. 1). Linn Run and Stone Run experienced severe acidic episodes, and Baldwin Creek and Benner Run acted as reference streams. We selected physically similar streams draining undisturbed forested watersheds of the northern Appalachian Plateau (Table 1). Baldwin Creek had a higher gradient and drained a smaller watershed than the other streams. Additional descriptions of streams and their watersheds are available elsewhere (Sharpe et al. 1984; DeWalle et al. 1989).

Brook trout were captured by electrofishing (700–900 V DC; Smith-Root model 12A) from headwater mountain streams (Table 2) similar to the study streams and from the study streams. Fish were held in cages in Baldwin Creek for 1–10 d before transmitter insertion for the fall 1988 and spring 1989 experiments. During the following two study seasons, fish were held in troughs (natural photoperiod) in the Black Moshannon Aquatic Research Laboratory for 2–6 d before transmitter insertion. Al concentration and pH of water in holding cages and troughs were similar to baseflow Al and pH of the study streams (Table 2).

Fish were assigned to study streams arbitrarily to ensure interspersion regarding source streams. Transmitters were placed in stomachs during the fall 1988 and spring 1989 seasons, but because we found several instances of regurgitation, we surgically implanted transmitters in the following two study seasons. In the first two seasons, each fish was anesthesized for 3 min in 15 L of 10 mg quinaldine/L Baldwin Creek water. The transmitter was then gradually pushed into the esophagus. Anesthesia for surgical implantation was 6 min in 15 L of 20 mg quinaldine/L Black Moshannon Lake water. Transmitters were placed in abdominal cavities through a 1- to 1.5-cm midventral incision that was then closed by three sutures. All surgical materials were sterilized in ethanol. Lengths of transmitter fish ranged from 153 to 243 mm.

Elapsed time between a fish's entering the anesthesia bath and being returned to recovery water ranged from 4 to 6 min

TABLE 1. Location and physical characteristics of the 200-m stream sections where transmitter-equipped fish were released. Watershed area was determined using 7.5′ U.S. Geological Survey topographic maps. All other variables were measured in the field on June 20–21, 1990. The last four variables are means.

	Linn Run	Baldwin Creek	Stone Run	Benner Run
Latitude (N)	40°09′	40°21′	41°06′	40°56′
Longitude (W)	79°13′	79°03′	78°27′	78°01′
Watershed area, km^2	10.0	5.4	11.6	11.3
Gradient, m/100 m	2.9	4.8	2.3	1.7
Bank width, m	6.6	5.3	5.1	4.8
Wetted width, m	5.6	4.6	4.6	4.4
Depth, m	0.14	0.12	0.21	0.19
Velocity, m/s	0.08	0.12	0.14	0.22

TABLE 2. Source drainage and number of fish that received transmitters. Fish were randomly assigned from source streams to study streams. Stream pH and Al levels are from dates of fish collections.

Year	Study streams (acidic vs. reference)	Source streams			
		Name	pH	Al, µg/L	*N*
Fall 1988	Linn Run vs. Baldwin Creek	Linn Run	6.6	15	3
		Baldwin Creek	6.9	14	6
		Roberts Run	6.6	22	4
		Stone Run	6.3	25	7
					(20)
Spring 1989	Linn Run vs. Baldwin Creek	Linn Run	6.0	50	2
		Roberts Run	5.4	109	12
		Cozy Hollow	—	—	15
		Six Mile Run	6.7	30	1
					(30)
Fall 1989	Stone Run vs. Benner Run	Linn Run	5.9	61	13
		Baldwin Creek	6.3	39	3
		Roberts Run	5.5	66	2
		Stone Run	5.8	52	6
		Benner Run	5.9	41	4
					(28)
Spring 1990	Stone Run vs. Benner Run	Six Mile Run	7.1	4	14
		Smays Run	7.0	3	2
		Shannon Run	6.4	4	1
		Tark Hollow[a]	5.4	24	3
					(20)

[a]Water sample collected January 21, 1988.

TABLE 3. Transmitter suppliers and sites of use.

Year	Study streams (acidic vs. reference)	Manufacturer	Model[a]
Fall 1988	Linn Run vs. Baldwin Creek	L.L. Electronics (Mahomet, Ill.)	LF-1-312-1
Spring 1989	Linn Run vs. Baldwin Creek	L.L. Electronics	LF-1-1/4L
		Wildlife Materials, Inc. (Carbondale, Ill.)	SOPI
Fall 1989	Stone Run vs. Benner Run	Wildlife Materials, Inc.	SOPI
		Wildlife Materials, Inc.	SOPI
Spring 1990	Stone Run vs. Benner Run	Wildlife Materials, Inc.	SOPI
		Wildlife Materials, Inc.	SOPI

[a]Transmitters were evenly divided among study streams to ensure interspersion relative to supplier. Slightly smaller transmitters (SOPI) were placed in smaller fish.

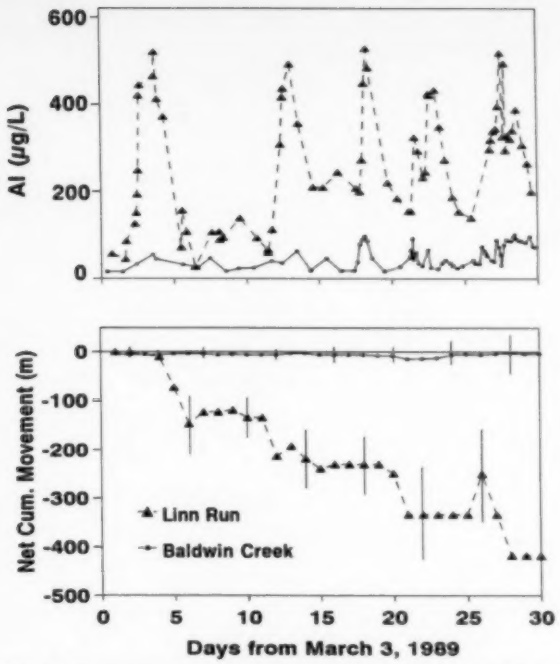

FIG. 2. Al concentrations and net cumulative movement of brook trout in Linn Run and Baldwin Creek during the spring 1989 study period.

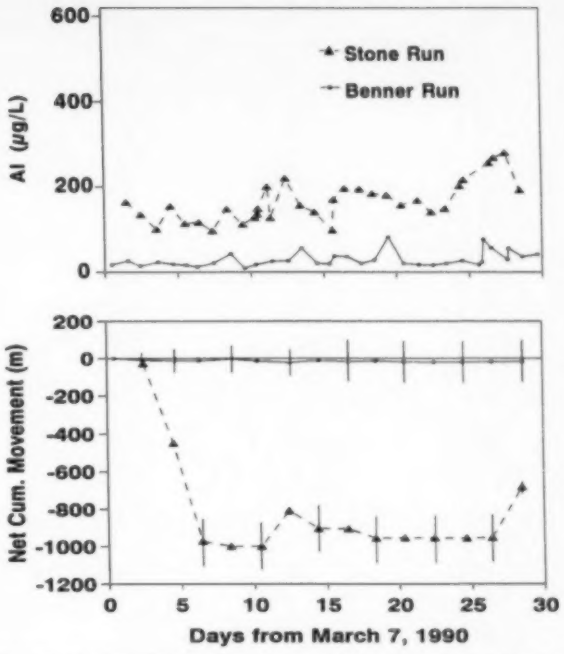

FIG. 3. Al concentrations and net cumulative movement of brook trout in Stone Run and Benner Run during the spring 1990 study period.

for stomach insertions and from 6 to 18 min for surgical implants. The fish were confined in holding cages for 1 d after insertion or in troughs for 3 d after surgical implantation before they were released in study streams. During these observation periods, an average of 6% (one or two) of the transmitter-equipped fish died or appeared abnormal. These were replaced.

The single-stage transmitters operated at unique frequencies between 164.000 and 166.000 MHz. The receiver (Telonics TR-2) and antenna (Yagi-4 element) combination could detect signals in situ at a distance of 100 ± 20 m, depending primarily on differences in transmitter design. Transmitter size and lifetime were also influenced by the manufacturer's design (Table 3); therefore, we assigned transmitters to study streams arbitrarily to ensure interspersion regarding manufacturer.

The fish with transmitters were released at approximately 10-m intervals in 200-m segments of the study streams. A continuous water quality and quantity monitoring station was in operation on each stream (additional description of the continuous monitoring stations is available in DeWalle et al. 1989). Water samples were collected by hand and by automated sampler (ISCO, 2700). Water level was recorded continuously (Belfort, FW-1PR) to characterize acidic episodes.

Fish were randomly assigned to streams within a season. Thus, the study design can be viewed as a comparative experiment between streams. It was not possible to ensure interspersion of fish source and transmitter source among seasons and this constitutes a limitation in the study design. Fish locations were determined daily or every other day for up to 30 d. Locations of individual fish were recorded as the distance (by pacing) from markers set at 100-m intervals. Differences between actual distances and those estimated by pacing ranged from 1 to 5 m for the four technicians who located all fish. Technicians could determine the actual location of a transmitter-equipped fish to within a circular area of 1 m radius. This allowed a sample of stream water to be collected within 1 m of each fish each time it was located. We located fish and sampled water on a daily basis during the first two seasons and every other day thereafter. We assumed that there was little change in water chemistry and did not collect a water sample if a fish was within 3 m of its previously sampled location and stream discharge had not changed. If two fish were within 3 m of each other, a single water sample collected at a point midway between them was used to characterize ambient chemistry for both of them. During the experiments conducted in fall 1988 and spring 1989 when a fish had moved more than 25 m from one day to the next, both its current and previous location were sampled.

Water samples were collected by hand in clean polyethylene bottles that were previously rinsed with distilled and deionized water and finally with water from the sampling location. Samples were refrigerated and returned for analysis to the Water Laboratory at the Environmental Resources Research Institute (at The Pennsylvania State University) within 72 h of collection. For samples collected at fish locations, we determined pH, Al concentration, and acid neutralizing capacity (ANC). For samples collected at the continuous monitoring stations, we determined pH, ANC, specific conductance, and all major cations and anions (except in fall 1989 when we only measured pH, Al, and Ca). We restrict data reported here to pH, ANC, Al, and Ca concentrations (concentrations of the other major cations and anions are reported in DeWalle et al. 1989). Chemical analy-

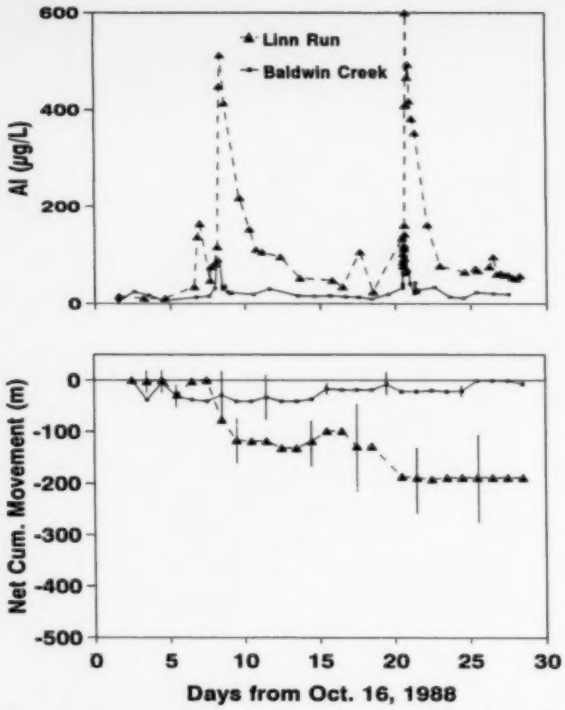

FIG. 4. Al concentrations and net cumulative movement of brook trout in Linn Run and Baldwin Creek during the fall 1988 study period.

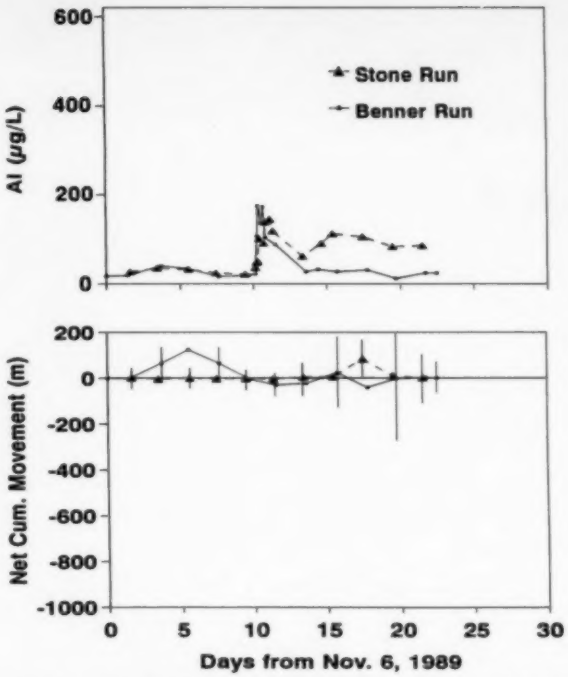

FIG. 5. Al concentrations and net cumulative movement of brook trout in Stone Run and Benner Run during the fall 1989 study period.

ses followed procedures of the USEPA (1983) except where noted. An Orion Ionalyzer and Ross combination electrode were used to measure pH. A Radiometer automatic titrater was used to measure ANC following Gran's method (Gran 1952). Dissolved Ca was determined by flame atomic absorption spectrophotometry on aliquots filtered through a 0.45-µm pore size millipore filter. Because Al-rich colloidal particles frequently pass through 0.45-µm pores (Kennedy et al. 1974), aliquots for Al determination were filtered through a 0.1-µm pore size millipore filter at 4°C and acidified before analysis by graphite furnace atomic absorption spectrophotometry. Therefore, Al concentrations reported in this paper can be described as total dissolved Al. Pagano (1990) determined concentrations of total monomeric Al, organic monomeric Al, and inorganic monomeric Al (by difference) for a subset of samples collected at the continuous monitoring stations. Further details on quality control and quality assurance are available elsewhere (Peck et al. 1988).

Results

Increased stream discharge was associated with increased Al concentration, especially in Linn Run and Stone Run. Al concentration at a given stream discharge was lower in the reference streams, Baldwin Creek and Benner Run, than in Linn Run and Stone Run. Stream pH ranged from 5.4 to 6.9 in Baldwin Creek versus 4.9–6.7 in Linn Run and from 4.9 to 6.2 in Benner Run versus 4.8–6.0 in Stone Run. Median Ca concentration (milligrams per litre) for each stream was as follows: Baldwin Creek, 3.4; Linn Run, 3.4; Benner Run, 1.7; Stone Run, 2.0.

Median net movement, relative to where each fish was initially released, was downstream for brook trout in Linn Run and Stone Run in spring (shown as negative values in Fig. 2 and 3). Al concentrations were higher in spring than in fall for both streams. We also measured two brief episodes of high stream Al concentration and net downstream movement of brook trout in Linn Run in fall (Fig. 4). While individual fish in the reference streams occasionally moved more than 100 m downstream in 2 d, these movements were offset by other individuals moving upstream. Thus, net movement remained near zero in the reference streams. Stream Al concentration was low and net movement was near zero for Stone Run in fall (Fig. 5). Net downstream movement in the acidic streams was primarily a result of reduced upstream movement rather than increased downstream movement relative to fish in the reference streams. Net downstream movement of fish coincided with the streams that had high Al concentrations and seasons of high Al concentration measured at the continuous monitoring stations.

The median net movements of fish in the acid and reference streams were tested statistically for significant difference using the Mann–Whitney test. For comparisons of fall data the medians were significantly different on only two of 38 days. For spring data, medians were significantly different on 29 of 44 days. The alpha level for all tests was ≤ 0.05. For the spring data, statistical comparisons were hampered in the later days of field exposure by mortality of transmitter fish which drastically reduced the sample size.

As they moved downstream, some fish moved into water of lower Al concentration than at the continuous monitoring station (Fig. 6). On a number of days, the median Al con-

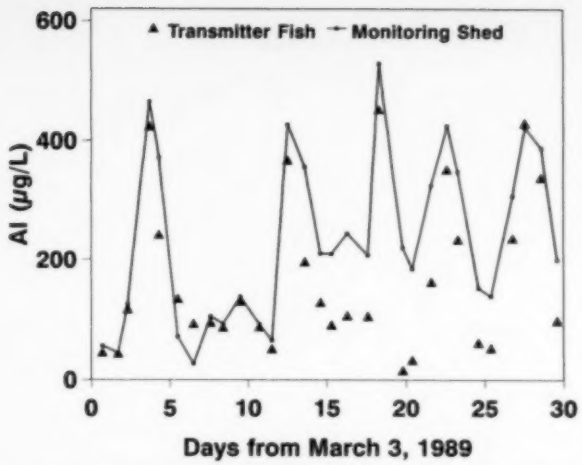

FIG. 6. Median Al concentrations at transmitter fish locations on Linn Run and comparable values for the stream at the water quality monitoring station.

centration at fish locations in Linn Run was over 100 µg/L lower than the Al concentration at the continuous monitoring station. Al concentration decreases in streams like Linn Run and Stone Run are due to inflow from relatively alkaline springs and tributaries (Sharpe and DeWalle 1990).

We documented limited movement of fish into more alkaline springs and tributaries. Initially, ambient Al concentrations for fish in Linn Run were similar to Al concentrations at the nearby continuous monitoring stations (Fig. 6). During the early stages of a severe acidic episode, most fish were subjected to Al concentrations similar to those of the mainstream. However, by day 4, 1 d after the onset of the episode, the median transmitter fish was exposed to a lower Al concentration than were fish in the mainstream (most fish were still exposed to over 200 µg Al/L; Fig. 7). Two fish were recovered dead on day 6. Prior to the next episode, on day 11, three of the 12 fish were in water of high ANC relative to the mainstream. During the episode on day 12, there were two fish in water with ANC in excess of 200 µeq/L whereas the remaining 10 fish were in water of less than 20 µeq/L (day 12; Fig. 7). One of these moved into an alkaline microhabitat less than 200 m downstream of the continuous monitoring station, and the other remained in the alkaline microhabitat that it had occupied previously. These two individuals and another fish were exposed to less than 100 µg Al/L whereas the remaining fish were exposed to over 300 µg Al/L. The other two fish that were in alkaline microhabitats on day 11 were exposed to high Al on day 12. Two fish were recovered dead on day 12 and another on day 15 for a total of five dead fish out of the 15 released. Few fish took full advantage of available alkaline microhabitats.

During the spring experiment in Stone Run, Al concentration was high, but relatively stable, generally between 100 and 300 µg/L (Fig. 3). Fish were initially released at sites with Al concentration and ANC similar to levels at the continuous monitoring station. Two days after release, many of the fish had moved to locations of lower ambient Al concentration relative to that at the continuous monitoring station (Fig. 8). ANC values at fish locations were low and remained similar to ANC at the continuous monitoring station. Stream

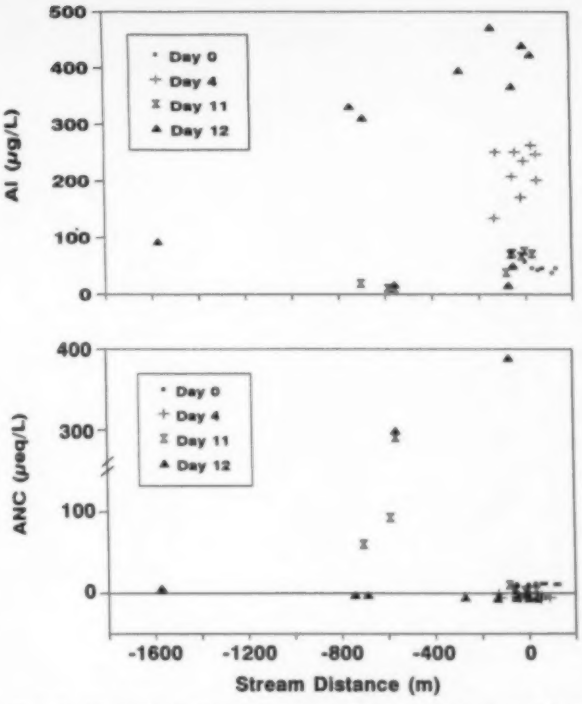

FIG. 7. Al concentrations at transmitter fish locations on Linn Run for four days during the spring 1989 study period.

chemistry remained relatively stable through day 8. By this time, nine of the 10 fish had moved over 400 m downstream and five of these were in water of less than 50 µg Al/L. Three fish, within 50 m of each other, were exposed to lower Al concentration than were two fish farther downstream (Fig. 8). Two fish were recovered dead on day 10 and another on day 16. Thus, three of the 10 transmitter-equipped fish were recovered dead. No dead fish were recovered from reference streams and we observed no mortality within the acidic streams in fall.

Discussion

Our data support the hypothesis that increased mortality rates due to acidic episodes diminish trout densities. Even greater losses due to net downstream movement would similarly diminish trout densities. Lack of upstream movement by individuals in the more acidic streams accounted for extensive net downstream movement in the present study. Downstream movement resulted in fish exposure to higher ambient pH and lower ambient Al concentration; however, because about one third of the fish died as they moved downstream during the spring episodes, it is apparently not a mechanism that ensures survival of fish during acidic episodes.

Brook trout are mobile and as long as stream gradients are not too high could recolonize stream segments that were vacated during acidic episodes. Gagen et al. (1989) reported that brook trout from a tributary rapidly colonized an upstream segment of Linn Run after the mainstream was neutralized by the addition of alkaline groundwater. However, most of the fish never moved more than 400 m upstream.

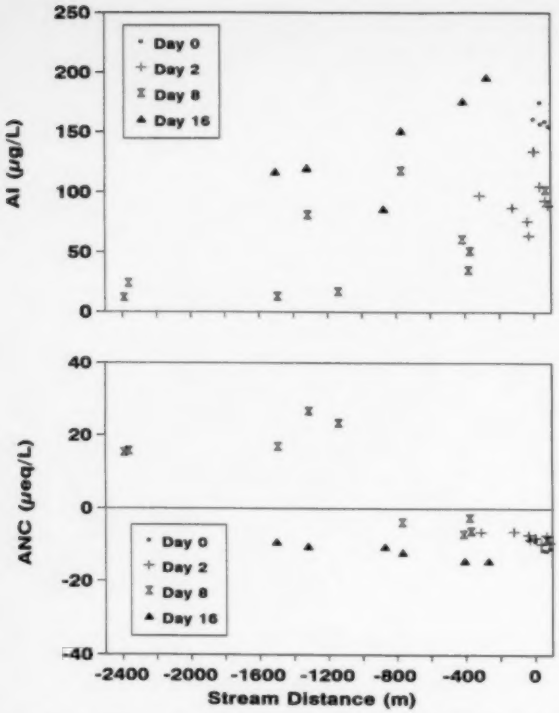

FIG. 8. Al concentrations at transmitter fish locations on Stone Run for four days during the spring 1990 study period.

There was no tendency for brook trout to move back into abandoned stream segments during the duration of our experiments; therefore, we consider downstream movement an important mechanism leading to population loss. With a longer study duration, brook trout may have recolonized after the extensive downstream movement. Recolonization seems to be a viable explanation for low densities of brook trout observed in streams like Linn Run (Gagen 1991).

Although this study was not specifically designed to determine whether downstream movement was directed or passive, we hypothesize that the latter is likely. Gagen et al. (1989) reported significant downstream movement of hatchery-reared brown trout (*Salmo trutta*) during an acidic episode that caused mortality of brown trout, but brook trout, a more acid-tolerant species, showed no response. Sublethal acidic episodes did not produce measurable movement by either species. We observed in this study that in Linn Run the greatest downstream movement was delayed more than 1 d after the onset of an episode. During an episode in fall 1988, transmitter-equipped fish in Linn Run moved downstream even though no mortality was observed in colocated bioassay cages (Gagen et al. 1993). However, Al reached concentrations reported in laboratory studies to be lethal and the caged fish appeared lethargic; consequently, passive downstream movement of fish was likely. A separate study, conducted during acidic episodes in spring 1988, demonstrated that a greater number of hatchery-reared brook trout remained in a section of Linn Run with an alkaline microhabitat than in an adjacent section without an alkaline microhabitat (Sharpe and Gagen 1993). However, considerable downstream movement also occurred as measured by traps located downstream of each section. This movement was also delayed relative to the onset of the episode. Mortality of free-ranging and caged fish confirmed that movement was concurrent with severe physiological stress.

The failure of any transmitter-equipped fish to move upstream to a small alkaline tributary to Linn Run also argues for passive movement. Telemetered fish were placed in Linn Run within 130 m downstream of this tributary, and all moved downstream from this point. This tributary was a gathering point for hatchery fish planted upstream of its confluence with Linn Run in previous experiments (Sharpe and Gagen 1993).

Passive downstream movement cannot be eliminated as a possible explanation for most reported field studies of fish congregating in alkaline microhabitats to avoid acidic episodes. Observations of apparently congregating salmonids have been generally unanticipated and in response to chemical conditions that could prove fatal (e.g., Leivestad and Muniz 1976; Hall et al. 1980; Skogheim et al. 1984). Most of these studies also reported mortality of free-ranging or caged fish (e.g., Muniz and Leivestad 1981; Watt et al. 1983). Even though Gloss et al. (1989) anticipated movement and had traps in place, water samples were not collected frequently enough to determine whether movement of brook trout from an acidifying lake (via outflow stream) was a directed or passive response. Hesthagen (1989) observed abnormal behavior including downstream drift in salmonids and attributed effects to severe stress during fatal acidic episodes in a Norwegian river. If avoidance behavior can mitigate effects of acidic episodes, we would have expected transmitter-equipped fish to have responded more quickly to changing water quality before lethal conditions occurred.

Other factors may have been important determinants of fish movement. Interactions between the telemetered fish and members of the resident population could have influenced the observed movement. However, it does not seem plausible that the larger telemetry fish would be displaced by smaller resident fish. It should be remembered that the largest available fish were selected for transmitter installation. Eighty-eight percent of the telemetry fish were in the 90th percentile or higher for length in the resident fish populations into which they were released. In addition, there was less net downstream movement in the fall than in the spring. This would seem to argue against displacement by resident fish because displacement should not change with season and in fact if anything should be greater in the fall when streamflows are lower with concomitant restriction of available habitat.

Fish kills have only rarely been linked to acidic episodes in streams in North America. This lack of evidence led Neville (1985) to hypothesize that fish escape death by seeking refuge areas during acidic episodes. Unfortunately, few studies have been designed to detect fish kills in streams and, where fish kills have been reported, researchers have emphasized the difficulties of documenting mortality (Skogheim et al. 1984; Gagen et al. 1989; Hesthagen 1989). The use of telemetry in the present study increased our chances of documenting mortality of free-ranging fish in conjunction with acidic episodes.

We cannot completely discount the possibility that handling stress and transmitter implantation artificially increased mortality rates relative to indigenous fish. However, the lack

of fish mortality in Baldwin Creek and Benner Run indicated that this was not the case. Indigenous fish may have been more tolerant of acidic episodes relative to transmitter-equipped fish due to acclimation or selection. Acclimation to sublethal Al concentrations, 150 μg/L at pH 5.2 for 10 wk, greatly increased brook trout tolerance of 3-d acidic pulses (333 μg/Al/L at pH 4.8) in studies by McDonald and Milligan (1988). Furthermore, different genetic populations of a single species can show differences in tolerance of low pH and high Al (Robinson et al. 1976; Rosseland and Skogheim 1987). However, our own bioassays with Linn Run fish do not indicate any clear difference in tolerance when compared with fish from Baldwin Creek (Gagen et al. 1993).

Toxicity could have been overestimated if a large proportion of the total dissolved Al was actually nontoxic due to organic chelation (Driscoll et al. 1980). Pagano (1990) reported that over 90% of the total dissolved Al in Linn Run and Stone Run was typically in a reactive inorganic form. Therefore, organic chelation of Al was probably of minor consequence in the present study.

Although brook trout were found in alkaline microhabitats during acidic episodes, high mortality and net downstream movement limit the efficacy of this behavior for maintaining brook trout populations in streams. Therefore, the potential for avoidance behavior in stream fish may not compromise the validity of models predicting field toxicity from laboratory data as hypothesized by Neville (1985). Clearly, laboratory-based toxicity models should attempt to incorporate the rapid, episodic chemical changes that can occur (e.g., Curtis et al. 1989). Another major hindrance to predicting presence or absence (or future loss) of populations, based on in situ chemistry and laboratory toxicity models, may be the presence and distribution of alkaline tributaries with high fish densities that could provide nursery areas for remnant populations (Gagen 1991).

This comparative study of brook trout movements in natural stream systems was artificial in several ways that may limit valid extrapolation to indigenous populations. The transmitter-equipped fish were transferred from their natal streams and it is possible that their lack of physical or chemical familiarity with the location of alkaline microhabitats led to reduced microhabitat use. Behavioral interactions with fish already present may have restricted access of transmitter-equipped fish to these microhabitats. Nonetheless, these data constitute an important link between the numerous surveys of water chemistry and fish occurrence and the several recent laboratory studies of fish behavioral responses to waters with low pH and high Al concentration.

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Risk of Predation and Its Influence on the Relative Competitive Abilities of Two Species of Freshwater Fishes

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Experiments were conducted with fathead minnow (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*) to determine whether their relative feeding rates were affected by risk of predation. Six groups of both species were observed foraging for food provided by an automated feeder while two parameters were manipulated: the presence or absence of a predator, yellow perch (*Perca flavescens*), and clear or turbid water. It was hypothesized that the armour and spines of brook stickleback would be of greatest benefit during a direct encounter with a predator. I attempted to simulate this condition with turbid water and predator present. Feeding rates of the two species were significantly different, with fathead minnow feeding at a greater rate than brook stickleback in the absence of a predator. In the presence of a predator the feeding rate of fathead minnow declined significantly whereas that of brook stickleback was unaffected. This resulted in similar feeding rates for the two species in the presence of a predator. Results demonstrate that risk of predation can alter the relative competitive abilities of two species in situations where one species possesses a morphological adaptation against predation. In the absence of predators, these traits may be detrimental in interspecific competition for food.

Des expériences ont été menées sur la tête-de-boule (*Pimephales promelas*) et l'épinoche à cinq épines (*Culaea inconstans*) afin de déterminer si leur taux d'alimentation relatif était affecté par le risque de prédation. Pour chacune des espèces, six groupes ont été observés pendant qu'ils cherchaient leur nourriture, fournie par un alimenteur automatique, tandis que l'on manipulait deux paramètres : la présence ou l'absence d'un prédateur, la perchaude (*Perca flavescens*), et la transparence ou la turbidité de l'eau. Notre hypothèse était que la cuirasse et les épines de l'épinoche à cinq épines lui seraient grandement utiles lors d'une confrontation directe avec un prédateur. Nous avons tenté de simuler ces conditions en eau trouble et en présence d'un prédateur. Nous avons observé des taux d'alimentation des deux espèces significativement différents, la tête-de-boule s'alimentant à un taux plus élevé que l'épinoche à cinq épines en l'absence de prédateur. En présence d'un prédateur, le taux d'alimentation de la tête-de-boule diminue significativement, tandis que celui de l'épinoche à cinq épines reste inchangé. On obtient ainsi des taux d'alimentation comparables pour les deux espèces en présence d'un prédateur. Ces résultats démontrent que le risque de prédation peut altérer la compétitivité relative de deux espèces, dans les situations où une espèce seulement est dotée d'une adaptation morphologique la protégeant de la prédation. En l'absence de prédateurs, ces caractéristiques peuvent nuire à la compétition interspécifique pour les aliments.

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The presence of predators can cause dramatic alterations in the behaviour of prey (for a review, see Lima and Dill 1990). However, not all prey respond similarly to a threat of predation. Differences in the relative willingness to risk exposure to predators have been observed between species (Brown et al. 1989; McLean and Godin 1989; Kotler et al. 1991; Abrahams and Healey 1993) and between sexes within a species (Abrahams and Dill 1989; Holtby and Healey 1990).

Behaviour or morphology that minimizes vulnerability to predators can allow a species to exploit niches that are too dangerous for other species. One such example exists in terrestrial communities. Brown et al. (1989) and Kotler et al. (1991) have demonstrated that some species of desert rodents are better able to exploit resources in open areas where they are vulnerable to aerial predators. They hypothesized that better-developed auditory bullae in these species is an adaptation that provides a superior ability to detect approaching predators, thereby increasing their probability of escape.

Teleost fishes possess diverse morphologies, ranging from streamlined bodies to bodies with armour and spines. Armour and spines are probably an adaptation to reduce vulnerability to predators (Hoogland et al. 1957) and therefore may allow more efficient exploitation of dangerous areas (McLean and Godin 1989).

Two mechanisms may be responsible for allowing fish to exploit dangerous areas. First, predators prefer to attack unarmoured prey. Reist (1980) demonstrated that a predatory northern pike (*Esox lucius*) preferred to attack morphs of the brook stickleback (*Culaea inconstans*) that had reduced armour compared with the fully armoured morph. Second, armoured fish need to rely less on antipredator behaviours (Benzie 1966; McLean and Godin 1989), providing them more time for other activities. Spending less time watching for predators or deferring a response to a predator is analogous to an alteration in the foraging–fleeing trade-off proposed by Ydenberg and Dill (1986).

These benefits of armour may come at a cost. Many armoured invertebrates have lower feeding, growth, or reproductive rates than similar species that do not possess armour

TABLE 1. Mean wet weight (g) and total length (mm) of the fathead minnow and brook stickleback in each of the six groups used in the study.

	Fathead minnow		Brook stickleback	
Group	Weight	Length	Weight	Length
1	1.25	52.7	2.68	57.3
2	0.56	41.7	0.48	37.3
3	0.72	45.3	1.01	44.8
4	0.59	38.3	0.59	36.2
5	0.87	42.3	0.70	45.7
6	0.71	44.7	0.85	45.7

(Dodson 1984; Havel 1987). Furthermore, the positive correlation between the degree of armour and spines possessed by threespine stickleback (*Gasterosteus aculeatus*) and the ambient level of predation risk (Gross 1978) suggests that this morphology is expensive. Such a relationship is expected because a phenotypic adaptation should be conserved when the fitness-related benefits exceed the cost. For some populations, this may no longer be true and could therefore lead to a reduction in armour.

Clearly, the net value of armour as measured by relative competitive abilities will be determined by predation risk. In the presence of predators, armour may render one species competitively superior whereas in the absence of predators, it may become inferior.

This mechanism may operate in aquatic communities that contain brook stickleback (armoured with plates and spines) and unarmoured fathead minnow (*Pimephales promelas*). These species are common to southwestern Manitoba; they are found in 57% of surveyed streams and lakes and occur together in 35% of them (Manitoba Department of Natural Resources, Fisheries Branch, unpublished data). Armoured brook stickleback are less susceptible to attack by a predator than fathead minnow (Robinson 1989). These species appear to be potential competitors because of overlap in their diet (both consume small aquatic invertebrates (Scott and Crossman 1973), although the elongated intestine of the fathead minnow allows it to include lower quality food items in its diet) and because they co-occur in the same microhabitat at the same time (M.V. Abrahams, unpublished data). Furthermore, enclosure experiments have demonstrated that the presence of fathead minnow reduces the growth rate of brook stickleback (M.V. Abrahams, unpublished data).

In the current study, I measured the relative feeding rates of fathead minnow and brook stickleback while manipulating two ecological variables: the presence of a predator and water transparency. These species are often found in turbid water and I hypothesized that water clarity will determine the distance at which prey can detect a predator (and vice versa) and the relative effectiveness of antipredator behaviours. Clear water will allow a predator to be detected while it may still be avoided. Under these conditions, the relative effectiveness of behavioural avoidance and antipredator morphology may be similar. However, in turbid water where the predator is only detected when avoidance is impossible, armour may be the more effective strategy.

The experiments reported in this paper test the prediction that antipredator morphology improves a species' competitive ability when feeding in the presence of a predator. Furthermore, I also tested the prediction that the relative benefit of armour should further increase in turbid water due to the reduced effectiveness of behavioural antipredator strategies under this condition.

Methods

For these experiments, juveniles fathead minnow and brook stickleback were collected by seine net and minnow trap from the marsh at the University of Manitoba's field station (located at the southern tip of Lake Manitoba, approximately 125 km west of Winnipeg) during the summer of 1991. These fish were taken to the University of Manitoba where they were held in 150-L fibreglass flow-through tanks and maintained on a diet of Tetra Min™ flakes and frozen brine shrimp (*Artemia salina*).

Experiments were conducted in a 200-L glass aquarium that was divided in half by a clear Plexiglas partition. A removable opaque partition covered the transparent partition. In one half of the aquarium, a feeder provided 0.5 g (wet weight) of freshly thawed adult brine shrimp delivered uniformly over a 24-min period (see Abrahams 1989 for a detailed description of these feeders). This feeder was located so that food was delivered 3 cm from the transparent partition and just below the surface of the water. The food immediately sank, unconsumed food collecting in a food trap beneath the feeder.

Six groups were used for these experiments. Three individuals from each species constituted each group. Individuals were selected so that mean sizes within a group (Table 1) were similar (within 5 mm total length). The six fish from each group were placed together in the apparatus and trained to use the feeder for 1 wk prior to beginning experiments. The feeder served as their only source of food.

Presence or absence of a predator and water turbidity were used to generate four different treatment combinations that were presented in a random order to each group. Foraging trials for a given fish group at a given treatment combination were conducted over 3 successive days, three times per day, with each trial separated by 3 h. Between trials, an opaque cover was placed over the transparent partition and was removed just prior to each trial. A 120-W halogen bulb shone light through an opening in a piece of matboard to illuminate the area under the feeder. This allowed the fish to be observed and videotaped under all treatment conditions with a High Band 8-mm video camera.

The predator used in the experiments was a 112-g (19.5 cm fork length) yellow perch (*Perca flavescens*). This fish was also collected at Delta marsh and was capable of consuming fathead minnow and brook stickleback in captivity. The yellow perch was placed behind the transparent Plexiglas partition in the experimental aquarium. Because of this partition, the experimental fish could not be captured by the predator but had to feed in close proximity to it. Turbid water was created by adding 3.8 mL of each of blue, red, and green food dyes to the aquarium. Using the untreated water as the blank, this treatment resulted in absorbance readings of 0.0034 at 450 nm, 0.0030 at 500 nm, and 0.0034 at 650 nm. The water in the aquarium was changed following the turbidity treatment.

Each trial lasted 24 min. To measure relative competitive abilities of the fish within each trial, I recorded from the video tapes the number of food items eaten by individuals of

TABLE 2. Summary of regression analyses to determine whether fish habituated to the treatment combinations. This was tested by determining whether the observed slope between the number of unconsumed food items and order within a treatment combination was significantly different from 0. Reduced error degrees of freedom for two treatments were due to missing data.

Treatment	Observed slope	Error df	F	P
No predator, clear	0.368	50	1.600	0.212
No predator, turbid	0.181	52	0.604	0.440
Predator, clear	−0.289	52	0.395	0.533
Predator, turbid	−0.578	50	0.662	0.420

TABLE 3. Summary of one-way repeated measures ANOVA to examine the effect of time within an experiment on the observed proportion of food consumed by each species for each treatment combination. Data were arc-sin square-root transformed prior to analysis. Degrees of freedom for all reported F-values are 2,10.

Treatment	F	P
No predator, clear	0.19	0.833
No predator, turbid	0.30	0.748
Predator, clear	0.95	0.420
Predator, turbid	2.33	0.148

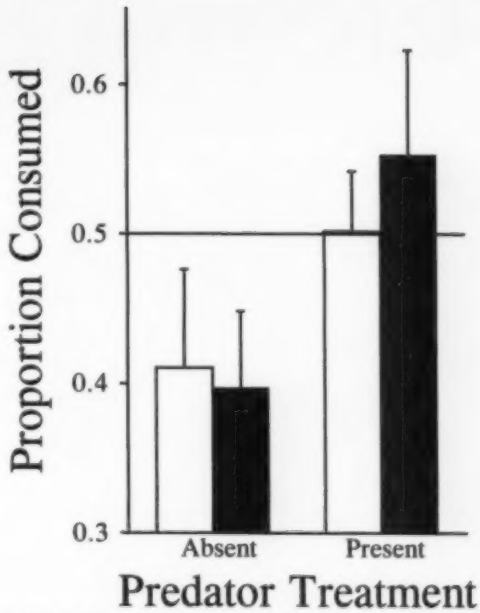

FIG. 1. Proportion of food consumed by brook stickleback under the four different treatment combinations used in this experiment. The open bars represent the clear water treatments and the shaded bars represent the turbid water treatments. Error bars correspond to 1 SE.

each species for the first, middle, and final 5-min sections of each trial. The average of these three observations represented the results for a single trial.

For each trial, I determined the proportion of food obtained by each species, and the amount of food that was not consumed. For statistical analysis, groups were considered as independent observations. Therefore, the mean of nine observations (three observations per day for 3 d) was used as a single observation to describe the results of each treatment combination for each group. Statistical analysis employed a randomized-block ANOVA with fish group as blocks.

Results

In these experiments, the test fish were exposed to the same treatment combination for nine sequential trials (three trials per day for 3 d). A concern with this experimental design was that the fish may habituate to a particular treatment combination, particularly since the predator was separated from the fish by a Plexiglas partition and therefore did not pose a real threat to the fish. The expected antipredator response would be avoidance of the feeder resulting in reduced feeding rates and an increase over time in the number of food items arriving in the food trap. Habituation should result in a sequential reduction in avoidance and therefore a sequential reduction in the number of food items in the food trap at the end of each trial. For each of the four treatment combinations, no sequential change was observed in the number of unconsumed food items (Table 2). Therefore, pooling data over the nine sequential trials per fish group is justified.

The proportion of food consumed by fathead minnow and brook stickleback during the first, middle, and final 5-min intervals was not significantly different (Table 3), indicating that the relative behaviour of the two fish species did not change through time within these experiments. Therefore, the mean value of these three observation periods should accurately describe the result of each experiment.

A further concern with this experiment was that the turbidity treatment would limit my ability to accurately observe the food items consumed by the two species. If this was the case, then the relationship between the total number of food items consumed and the number collected in the food traps should differ between the turbid and clear water treatments (e.g., reduced feeding rates in the turbid water treatment would be due to an inability to observe all food items consumed, not an actual reduction in feeding rate). A comparison of the regression equations for this relationship yielded no significant difference in the slope ($F_{1,104} = 2.268$, $P = 0.135$) or the intercept ($F_{1,104} = 0.078$, $P = 0.78$). Therefore the turbid water treatment did not impair my ability to accurately observe feeding behaviour in these experiments.

The presence or absence of the predator had a significant influence on the relative amount of food captured by each fish species (two-way ANOVA; $F_{1,15} = 4.85$, $P = 0.0437$). In the absence of the predator, brook stickleback obtained 40.4% (±4% SE) of the consumed food, which was significantly less than that consumed by fathead minnow (Fig. 1). Adding a predator to the apparatus increased the brook stickleback's share of consumed food to 52.7% (±4% SE), which was not significantly different from the consumption rate of fathead minnow (paired t-test, $t_5 = 0.576$, $P > 0.50$). Varying the turbidity of the water had no effect on the relative feeding rates of the two species (two-way ANOVA; $F_{1,20} = 0.17$, $P = 0.68$), nor was there an interaction between these two treatments (two-way ANOVA; $F_{1,20} = 0.32$, $P = 0.58$). The

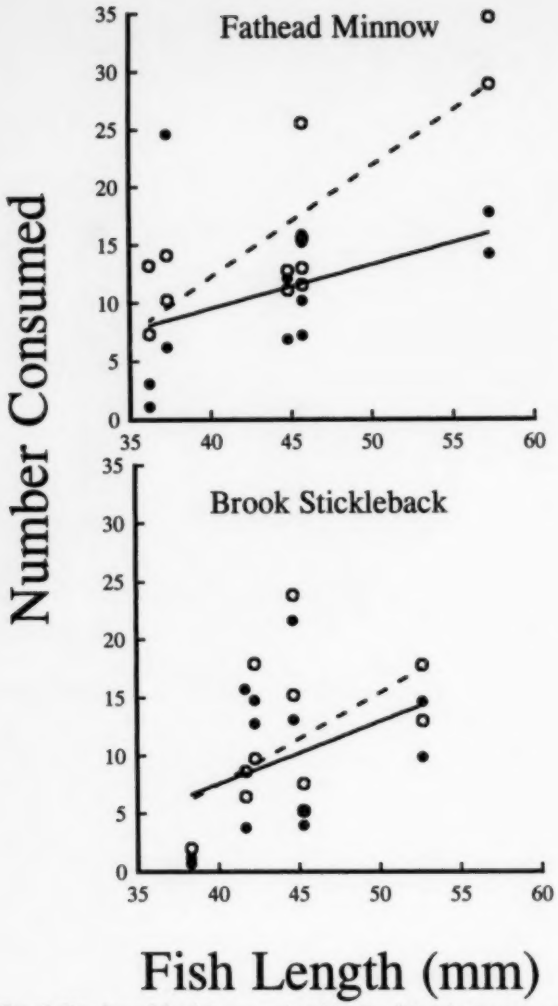

FIG. 2. Number of food items captured by the fathead minnow and brook stickleback as a function of their size and the presence (solid circles) or absence (open circles) of a predator. Data have been pooled for both turbidity treatments. The broken and solid lines indicate the lines of best fit for experiments with no predator present and with a predator present, respectively.

blocking factor (group) had no significant influence on this result ($F_{5,15} = 1.09$, $P = 0.4036$).

For both species, the absolute number of food items captured differed significantly among groups (two-way ANOVA; fathead minnow: $F_{5,12} = 4.54$, $P = 0.015$; brook stickleback: $F_{5,12} = 8.96$, $P = 0.001$). However, the presence of the predator only affected the feeding rate of fathead minnow (Fig. 2). In the presence of the predator, their absolute feeding rates declined significantly (two-way ANOVA; $F_{1,12} = 5.42$, $P = 0.038$). The number of food items captured by brook stickleback did not change in response to this treatment (two-way ANOVA; $F_{1,12} = 0.34$, $P = 0.569$). Therefore, the relative improvement in the food rate of brook stickleback in the presence of a predator was not due to an increase in their absolute feeding rate, but to a reduction in the absolute feeding rate of fathead minnow.

Discussion

These data demonstrated that the relative feeding rates of fathead minnow and brook stickleback were affected by the presence of a predator. In the absence of a predator, fathead minnow consumed proportionately more food than brook stickleback. Adding a predator resulted in both species consuming approximately similar quantities of food. Variation in the response to risk of predation appears to be the mechanism responsible for the change. In these experiments, fathead minnow were sensitive to risk of predation and reduced their feeding rate in the presence of the predator. Brook stickleback did not appear to modify their foraging behaviour in response to risk of predation resulting in feeding rates similar to fathead minnow in the presence of a predator.

The spines and armour of brook stickleback may benefit them in encounters with predators and therefore allow them to minimize their behavioural modification in response to the risk of predation. Reduced vulnerability to predators should allow brook stickleback to postpone their response and therefore not have to forego as many feeding opportunities. Indeed, McLean and Godin (1989) have demonstrated that fishes with increasing amounts of defensive armour are less likely to flee and reduce their reactive distance to an approaching predator. With direct encounters, the predator is detected at a distance requiring an immediate response by the prey. The armour and spines provide brook stickleback a finite probability of escape even if captured by the predator. In the absence of these defenses, I assume there would be no probability of escape. For this reason, I assumed that the combination of turbid water and the presence of a predator would be of the greatest relative benefit to brook stickleback. Manipulating water turbidity had no significant influence on the relative competitive abilities of these two species. Therefore, a postponed response to a predator is likely to be the most important reason for the shift in relative competitive abilities.

Werner (1991) has also demonstrated that competitive abilities can be altered by the presence of a predator. He examined the growth rates and competitive abilities of two species of anuran larvae (*Rana catesbeiana* and *R. clamitans*). In the absence of a predator, both species grew at similar rates and were similar in competitive abilities. However, in the presence of predatory odonate larvae (*Anax junius*), *R. catesbeiana* became competitively superior and achieved higher growth rates than *R. clamitans*. The interaction between brook stickleback and fathead minnow is the reverse of this system. Rather than the risk of predation causing an asymmetry in competitive abilities, it generates equivalence. Therefore, the presence of brook stickleback in some aquatic ecosystems may be dependent on the presence of predators.

Persson (1990) has also observed that predators reversed the relative competitive abilities of roach (*Rutilus rutilus*) and European perch (*Perca fluviatilis*) by altering their use of habitats. In the absence of predators, both species occupy open-water areas, where roach are competitively superior. However, in the presence of predators, juveniles of both species were forced into structurally complex refuges. In this habitat, the swimming performance of roach was impaired, resulting in a reversal of competitive abilities. In the absence of a predator-free control, it is difficult to deter-

mine whether a similar situation applies to the fathead minnow and brook stickleback in this study. However, both species were collected in open water in an environment that contains predators, suggesting that the size class of individuals used in these experiments does not shift habitats in response to predation risk.

Possession of traits specific to reducing susceptibility to predators must have some cost, and this may contribute to polymorphisms observed in threespine stickleback populations (for a review, see Bell 1976). Several potential costs have been proposed. Reimchen (1980) and Reist (1980) have demonstrated that spines make sticklebacks more susceptible to capture by predatory grasping invertebrates. Nelson (1969) suggested that elimination of body armour allows for streamlining and more efficient escape responses through dense vegetation. If these hypotheses are true, animals that possess morphological antipredator adaptations suffer from bearing these traits in the absence of predators. Only in the presence of predators should these traits confer any advantage to their bearer.

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Zooplankton Assemblages in the Ohio River: Seasonal, Tributary, and Navigation Dam Effects

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Ohio River zooplankton were collected monthly or quarterly for 1 yr from littoral and pelagic (main channel) areas in three navigation pools (constricted and floodplain) with four intrapool locations (lower pool and above, below, and within major tributaries). Total densities were minimal (1.64/L) when discharge and turbidities peaked (December–April) but were relatively high otherwise (21.63/L). Seasonal rises in rotifer density preceded increases in cladocerans and copepods; rotifers were nearly twice as abundant as cladocerans and copepods. Diversity (species richness) was not correlated with temperature, velocity, or turbidity. Density was positively linked with temperature and negatively correlated with river velocity and turbidity. Diversities in littoral and pelagic areas were not different, but densities were higher nearshore (mostly copepods and cladocerans); rotifers were usually more common in the main channel. Neither proximity to low-head navigation dams nor channel morphology had detectable effects on zooplankton. Although total diversity and density were similar in tributaries and the main channel, rotifers were more abundant in tributaries, and the Ohio contained more cladocerans and copepods. Densities in the Ohio were greater below than above tributaries for rotifers and cladocerans but lower for copepods, primarily because of nauplii.

Le zooplancton de la rivière Ohio a fait l'objet de prélèvements mensuels ou trimestriels pendant 1 an dans des régions littorales et pélagiques (chenal principal) dans trois bassins de navigation (étranglement et plaines inondables) avec quatre emplacements à l'intérieur du bassin (bassin inférieur et au-dessus, en dessous et à l'intérieur des principaux tributaires). Les densités totales sont minimales (1,64/L) lorsque le débit et la turbidité sont au maximum (décembre à avril), mais relativement élevées dans les autres cas (21,63/L). Les augmentations saisonnières de la densité des rotifères précèdent les augmentations de la densité des cladocères et des copépodes. L'abondance des rotifères est près de deux fois plus élevée que celle des cladocères et des copépodes. Aucune corrélation n'a été observée entre la diversité (richesse des espèces) et la température, la vitesse ou la turbidité. On a observé une corrélation positive entre la densité et la température et une corrélation négative entre la densité et la vitesse du cours d'eau et sa turbidité. Aucune différence n'a été observée sur le plan de la diversité entre les régions littorales et pélagiques; cependant, les densités sont plus élevées près du rivage (pour l'essentiel, copépodes et cladocères). Les rotifères sont en général plus répandus dans le chenal principal. Ni la proximité des barrages de canalisation à faible hauteur de charge, ni la morphologie du chenal ne semblent avoir d'effet détectable sur le zooplancton. Si l'on a observé des valeurs similaires pour la densité et la diversité totale dans les tributaires et dans le chenal principal, les rotifères sont cependant plus abondants dans les tributaires, tandis que l'on retrouve davantage de cladocères et de copépodes dans la rivière Ohio. Les densités observées dans la rivière Ohio sont plus élevées en aval qu'en amont des tributaires pour les rotifères et les cladocères, mais plus faibles pour les copépodes, principalement à cause des larves de nauplius.

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Large rivers have not been examined in an ecosystem context nearly as often as lakes or headwater streams; consequently, they represent ecological "black boxes" from a functional perspective (Thorp 1992a). Indeed, only about 4% of all studies conducted on flowing waters have dealt with large (greater than sixth order) rivers (Hynes 1989), and much of this research has concerned the distribution and relative abundance of fish and unionid mussels. Scientific studies of riverine plankton, or "potamoplankton," at organismal, population, and community levels are particularly rare. Of the relatively few papers published on riverine zooplankton, most report spatial and temporal community patterns in relation to hydrodynamics (e.g., Rossaro 1988; Saunders and Lewis 1988a, 1988b; Ferrari et al. 1989; Pace et al. 1992), hydrographic gradients (e.g., De Ruyter van Steveninck et al. 1990), suspended inorganic sediment and organic matter concentrations (e.g., Moghraby 1977; Shiel 1979), seasonal water temperatures (e.g., Neitzel et al. 1982; Rossaro 1988), and/or phytoplankton abundances (e.g., Shiel 1985).

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One focal area of process-oriented research in large tropical and temperate rivers has been an analysis of sources and fates of energy and nutrients. An early comprehensive view of energy flow was embodied in the river continuum concept (RCC) (Vannote et al. 1980; Minshall et al. 1983, 1985). The RCC stated that streams should be viewed as longitudinally interlocked systems in which ecosystem dynamics of downstream reaches are linked with processes occurring upstream. Biotic assemblages, such as the potamoplankton and riverine benthos, were thought to follow an orderly longitudinal shift in community composition primarily in response to upstream processes and changing stream morphology. Recent studies have concluded, however, that allochthonous organic matter derived from sources other than upstream processes (Junk et al. 1989) as well as autochthonous primary production and direct riparian inputs (Thorp and Delong 1994) are of crucial importance to large river food webs. In systems where extensive river–floodplain interactions occur, productivity of riparian vegetation and organic processing within floodplains may greatly modify longitudinal patterns of ecosystem processes predicted by the RCC (Junk et al. 1989). While it is increasingly recognized that large floodplain rivers do not conform to predictions of the RCC (Sedell et al. 1989), the applicability of this pivotal theory to large rivers with constricted channels is still debatable (Thorp and Delong 1994).

A major impediment to studying large rivers is that long, relatively pristine, and unregulated reaches of very large rivers have nearly vanished from developed nations and are rapidly disappearing from less-developed countries as a result of impoundment, channelization, and other regulatory processes undertaken for flood control, electrical power generation, water storage, navigation, and recreation (Benke 1990; Thorp 1992a). Effects of high-head dams on lotic ecosystems are relatively well known because of extensive research on reservoirs and the rivers flowing from them. Their influences depend partially on the dam's location along the river continuum and on factors such as the dam's height and water-release pattern (see discussion of serial discontinuity concept in Ward and Stanford 1983). In contrast, ecological effects of low-head navigation locks and dams, such as those on the Ohio and upper Mississippi rivers, are poorly known.

Some regulated rivers such as the Ohio are subdivided by lowhead dams into "pools." Despite their name, pools bear little resemblance to lakes, have seasonally high current velocities, and are not equivalent to the pool–riffle sequence of shallow streams (for a comparative review of lake and river attributes, see Ryder and Pesendorfer 1989). Current velocities, depth, suspended sediment concentration, and substrates vary from upper to lower portions of pools (Thorp 1992a), with likely effects on zooplankton residing in channel and nearshore areas. Many pools within the Ohio receive potentially important organic and inorganic contributions from tributaries. The structure and function of potamoplankton communities within these pools are presumably modified to some degree by inputs of drifting animals and phytoplankton, particulate organic matter (POM) and dissolved organic matter (DOM), inorganic sediment, and local differences in water quality. The impacts of these factors probably vary seasonally and may be influenced by the relationship between the wetted channel and its catchment (e.g., whether the pool resides within a constricted or floodplain region).

As part of a broader study of the Ohio River ecosystem, we investigated seasonal dynamics of potamoplankton in three pools of the Ohio River: two entirely within a constricted reach and one mostly confined to a floodplain segment. Our principal objectives were to determine seasonally (a) whether potamoplankton assemblages in the deepwater channel are significantly different from those near the banks, (b) effects of dams (partially by identifying community patterns along a gradient from the upstream to the downstream dam), (c) influences of major tributaries on potamoplankton, and (d) whether plankton assemblages differ in constricted-channel and floodplain regions of the river.

Methods

Study Sites

Based on mean annual discharge, the Ohio River is the second largest river in the United States, surpassed only by the lower Mississippi (below its confluence with the Ohio at Cairo, Ill.). The average annual discharge of the Ohio at Louisville (which is 604 km from the river's mouth) is 3273 m^3/s (115 500 ft^3/s), with a historical (63 yr of data) daily range of 59–31 432 m^3/s (McClain et al. 1993). It is divisible into (1) a region of constricted channel and relatively narrow floodplains (constricted by former glacial activity) which characterizie approximately two thirds of the river (from Pittsburgh, Pa., to just below Louisville, Ky.) and (2) a floodplain region from just below Louisville to the confluence of the Ohio River and the smaller, middle Mississippi River (Ray 1974).

Data were collected from three pools along a 361-km stretch of the Ohio (Fig. 1): the 155-km-long Markland Pool (most upstream reach), the 119-km McAlpine Pool, and the 185-km Cannelton Pool (most downstream reach). The Markland and McAlpine pools have relatively constricted channels with narrow floodplains generally alternating between right and left banks. The upper portion of the Cannelton Pool (near Louisville) is also constricted, but the pool gradually becomes a floodplain river below its confluence with the Salt River and has wide floodplains near the Cannelton dam.

In addition to studying the Ohio River mainstem, we took samples from the largest tributary entering each pool: the greater Miami River in the Markland Pool, the Kentucky River in the McAlpine Pool, and the Salt River in the Cannelton Pool (Fig. 1). The Miami River flows generally south and historically has a mean annual discharge near its mouth of 93 m^3/s. Both the Kentucky and Salt rivers flow northwest and have average annual discharges of 239 m^3/s (31 yr) and 51 m^3/s (6 yr), respectively.

Data on the species composition, diversity (species richness), and density of potamoplankton were collected monthly (McAlpine Pool) or quarterly (Cannelton and Markland pools) for 1 yr beginning in February 1991 from 24 sites (Fig. 1). Samples were taken from littoral (nearshore) and main channel (pelagic) areas in three navigation pools (constricted and floodplain) with four intrapool locations (lower pool and above, below, and within three major tributaries). The lower portion of each pool was sampled 8–16 km above the dam; samples in the upper portion of each pool were taken approximately 3 km above and below the Ohio's confluence with a major tributary. Zooplankton were also collected within the tributary itself at a distance of about 3 km

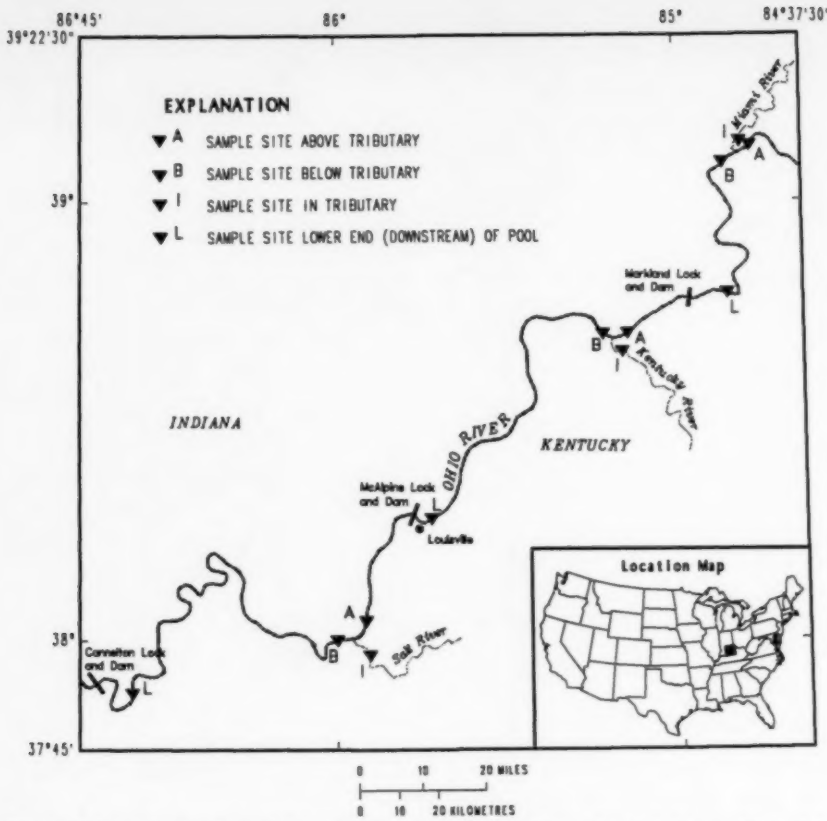

FIG. 1. Schematic drawing of the study area in the Ohio River showing the position of the three dams, pools, and tributaries and the collection sites within each pool. The Ohio changes from a constricted-channel river to a floodplain river just downstream of the Salt River.

upstream from its mouth. This provided information on the influence of both dams and tributaries on Ohio River potamoplankton. Samples of potamoplankton, along with physical data, were collected in a midchannel area and near the bank (1 ± 0.5 m in depth) at all sample sites. This afforded information on potentially distinct populations of littoral and pelagic potamoplankton.

Sample Methods

On each sample date, five zooplankton samples were collected from each of eight sites/pool, producing a total of 800 zooplankton samples. We obtained quantitative samples from a depth of 0.25 m with multiple dips of a 5-L bucket; sample volume (10–20 L) was varied monthly depending on zooplankton relative abundance. After pouring the water sample through a 63-μm net, the trapped zooplankton were transferred to 60-mL bottles, killed using hot water, and preserved with a modified Lugol's solution. Depending on the absolute abundance of zooplankton (especially nauplii), samples were either entirely identified or, more commonly, subsampled with a plankton splitter. A stereomicroscope and inverted compound microscope were used for sorting and identification.

Various physical and chemical measurements were taken at 0.5–1 m depth to characterize sample sites during each collection period. Current velocities in the channel and nearshore areas were measured with a Marsh McBirney velocity meter (model 201-D), and water temperatures were recorded with a YSI temperature probe. Water samples were retrieved for turbidity analysis from the 12 midchannel sites and measured in NTU units with a Hach turbidimeter (=nephelometer) standardized with known samples. Water samples for chemical analysis were filtered in line (Whatman GF/F) and frozen (−15°C) for later processing. Samples were analyzed for concentrations of soluble-reactive phosphorus (SRP) using the antimony–ascorbate–molybdate method (APHA 1985; Bran+Luebbe Analyzing Technologies 1986a). NH_4^+-N concentrations were measured using phenol–hypochlorite, and NO_3^- concentrations were quantified (after reduction to NO_2^- in a Cd–Cu column) via reaction with sulfanilamide–NNED (APHA 1985; Bran+Luebbe Analyzing Technologies 1986b, 1987a). Soluble reactive Si (as SiO_2) was measured via the molybdosilicate method (APHA 1985; Bran+Luebbe Analyzing Technologies 1987b), and SO_4-S was analyzed using the $BaCl_2$ method (APHA 1985; Bran+Luebbe Analyzing Technologies 1989). Methods were modified for automated analysis and run on a Traacs 800 automated analyzer (Bran+Luebbe Inc.). Na was measured using flame atomic absorption spectroscopy (Perkin-Elmer model 1100B).

Statistical Analyses

Most data analyses employed nonparametric statistical procedures because normality tests in the SAS program

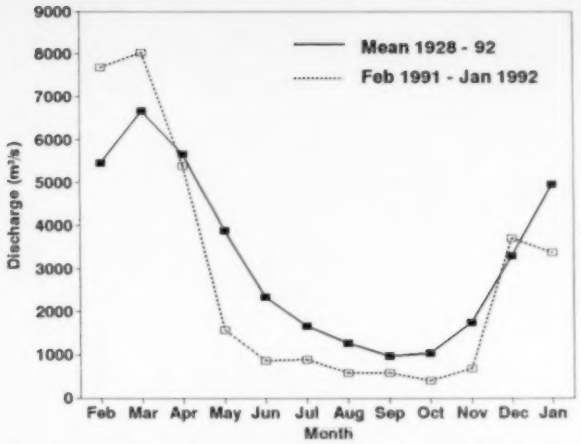

FIG. 2. Average monthly discharge of the Ohio River at Louisville (McAlpine dam) for the sample period (February 1991 – January 1992) and for the period of record (1928–92). (Data from the USGS Water Data Report for Kentucky, Water Year 1992).

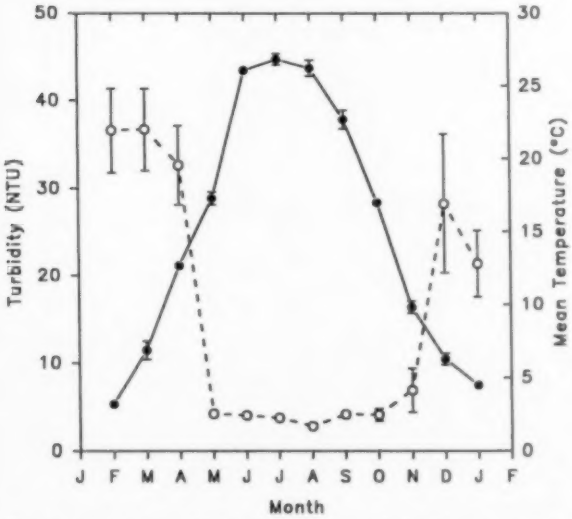

FIG. 3. Average monthly turbidity (suspended sediment concentration in NTUs) and water temperatures for the Ohio River during February 1991 – January 1992.

(SAS/STAT 1989) indicated that most data were not normally distributed, and this nonnormality was not sufficiently modified by several transformation techniques. Statistical procedures used to examine environmental effects were either nonparametric analysis of variance or Spearman's rank correlation coefficients. The alpha level used in all statistical tests to indicate significance was $P \leq 0.05$.

Results

Site Characteristics

Current velocities during the year-long study were greatest in the late winter and early spring and lowest in late summer. The maximum velocity we measured at 1 m depth in the

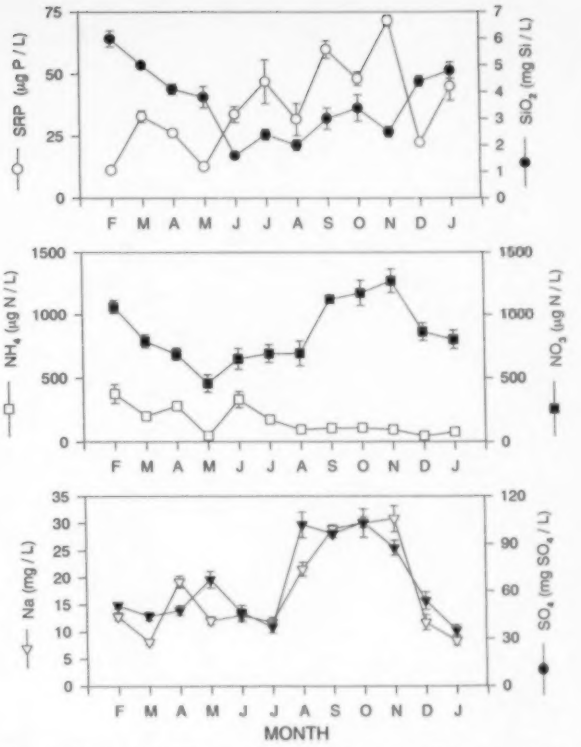

FIG. 4. Mean (±1 SE) monthly chemistry conditions in river water collected from the midchannel of the Ohio River in the McAlpine Pool during February 1991 – January 1992) (n = 15).

Ohio River was 1.7 m/s in midchannel. Velocities in the channel were always greater than those nearshore, which averaged <0.10 m/s but peaked at 0.55 m/s. Average monthly discharge patterns at the McAlpine dam for the period of our study and for the period of record (1928–92) are similar (Fig. 2), with high discharge in winter and early to midspring followed by a precipitous drop in late spring through mid-autumn. This two-phase pattern of discharge is reflected in the monthly patterns of suspended sediment concentration and water temperature (Fig. 3). Nearly all the winter–spring turbidity maximum is associated with resuspended sediments and is relatively uninfluenced by phytoplankton abundance (J.D. Wehr et al., unpublished data). River water chemistry also changed considerably during the year (Fig. 4). Most key variables varied inversely with discharge, especially SRP, NO_3, and SO_4. Maximum levels of these nutrients were observed during late summer and early autumn and declined in December (first substantial rise in discharge); however, SiO_2 concentrations decreased during summer low-flow periods and were greatest during the winter.

Seasonal Dynamics of Potamoplankton

Species richness, total zooplankton density, and densities of rotifers, cladocerans, adult copepods, and nauplii varied among months ($P < 0.01$ in all groups). Although species richness differed among months, consistent seasonal patterns were not evident. In contrast, patterns of zooplankton density corresponded well with the two-phase model of discharge

TABLE 1. Densities (no./L) of the most common zooplankton from the McAlpine Pool of the Ohio River. Data were compiled from three main channel sites sampled monthly during 1991–92.[a] n = 190 in Phase I and 277 in Phase II.

	Phase I (Dec.–Apr.)[a]			Phase II (May–Nov.)			Annual	
Taxon	Mean	SE	Max.	Mean	SE	Max.	Mean	SE
Cladocerans[b]	0.136	0.048	9.00	2.878	0.319	43.60	1.762	0.200
Bosmina	0.077	0.009	1.10	2.406	0.298	37.20	1.458	0.185
Ceriodaphnia	0.001	0.001	0.10	0.150	0.036	8.00	0.089	0.021
Daphnia	0.005	0.001	0.10	0.034	0.007	0.80	0.022	0.004
Rotifers[c]	0.390	0.031	2.10	11.872	3.188	814.40	7.201	1.908
Ascomorpha	0.018	0.003	0.20	0.001	0.001	0.25	0.008	0.001
Brachionus	0.076	0.014	1.20	0.001	0.001	0.20	0.032	0.006
B. calyciflorus	0.011	0.003	0.25	0.647	0.171	38.30	0.388	0.103
B. rubens	0.009	0.003	0.25	0.012	0.004	0.60	0.011	0.002
Euchlanis	0.050	0.011	1.15	0.086	0.026	4.00	0.071	0.016
Keratella	0.058	0.008	0.95	0.255	0.111	21.60	0.175	0.066
K. quadrata	0.002	0.001	0.15	0.231	0.218	60.40	0.138	0.129
Polyarthra	0.039	0.005	0.40	2.574	0.492	68.40	1.543	0.297
Copepods[d]								
Calanoida	0.038	0.008	0.80	1.360	0.209	37.60	0.823	0.127
Cyclopoida	0.187	0.015	1.15	0.483	0.055	9.60	0.363	0.034
Nauplii	0.712	0.046	3.10	11.719	1.547	188.80	7.241	0.951

[a]Phase I consisted of data from February, March, April, and December of 1991 and January and February of 1992; Phase II was May–November of 1991.

[b]Other cladocerans collected: *Diaphnasoma*, *Eubosmina*, *Moina*, *Simocephalus*. In the summer of 1992, large populations of the exotic species *Daphnia lumholtzi* were collected.

[c]Other rotifers collected: *Asplanchna*, *A. priodonta*, *A. brightwelli*, *B. bidentata*, *B. caudatus*, *B. havanaensis*, *B. quadridentata*, *B. urceolaris*, *Conchiloides*, *Filinia*, *K. cochlearis*, *K. testudo*, *Lecane*, *Nothalca*, *Platyias*, *Pleosoma*, *Rotaria*, *Syncheata*.

[d]Copepods consisted primarily of *Eurytemora* (Calanoida) and *Diacyclops* (Cyclopoida).

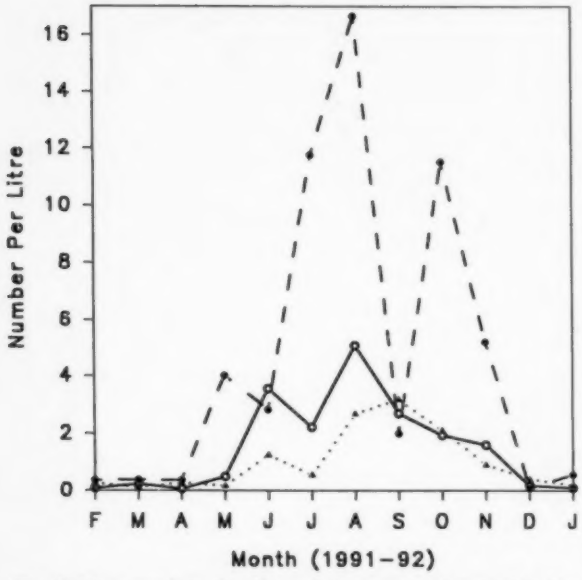

FIG. 5. Mean number of rotifers, cladocerans, and copepods during the study period. Solid circles and dashed line = rotifers; open circles and solid line = cladocerans; solid triangles and dotted line = copepods.

described above (Fig. 5). During the period when discharge and suspended sediment concentration are high and temperatures are low (Phase I: December–April in our sample year), average zooplankton densities were low (1.6/L). In contrast, during Phase II (May–November in our sample year), densities were relatively high (21.6/L, with a maximum monthly density of 34.6/L). Although a portion of this effect may have been due to dilution (mean monthly discharge was roughly six times greater in Phase I than in Phase II), a considerable portion of the disparity in densities between Phases I and II was due to differences in absolute population size. The seasonal patterns of abundance for major groups were similar to total density and to each other; however, a seasonal rise in density was first observed for rotifers, then for cladocerans, and lastly for copepods (Fig. 5). Of the 14 most abundant taxa, 13 were highly variable over time ($P < 0.0001$) and the fourteenth was almost significant ($P = 0.07$). All 14 taxa were most abundant during Phase II (Table 1).

As a group, rotifers were nearly twice as abundant as either cladocerans or copepods (exclusive of nauplii) and typically represented more than 50% of the individuals in the community (Table 1). The rotifer genus *Polyarthra* (principally *P. vulgaris* and *P. dolichoptera*) was the most abundant taxon on an annual basis; however, the next three numerically dominant taxa were the cladoceran *Bosmina longirostris*, calanoid copepods (mostly *Eurytemora affinis*), and cyclopoid copepods (primarily *Diacyclops thomasi*). All other numerically dominant zooplankton (defined here as present at >0.1/L) were rotifers: *Brachionus calyciflorus* and *Keratella* spp. (*K. quadrata* and several other species). *Polyarthra* spp. and *Brachionus* spp. were more abundant early in Phase II whereas *Keratella* spp. were more plentiful

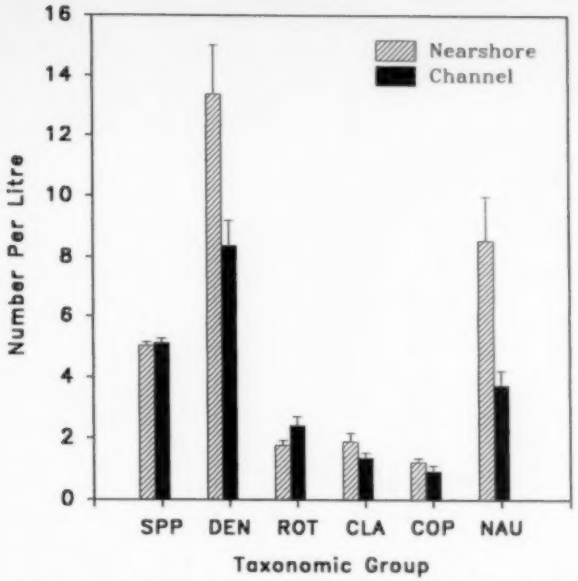

FIG. 6. Mean number of species (SPP) and individuals (±1 SE) in shallow nearshore areas (depth = 1 ± 0.5 m) (hatched bars) and in deeper midchannel areas (solid bars) of the Ohio River. In this and subsequent graphs, DEN = total zooplankton density (including copepod nauplii) and ROT, CLA, COP, and NAU = densities of rotifers, cladocerans, copepods (excluding nauplii), and nauplii, respectively. Data drawn from the Ohio River only.

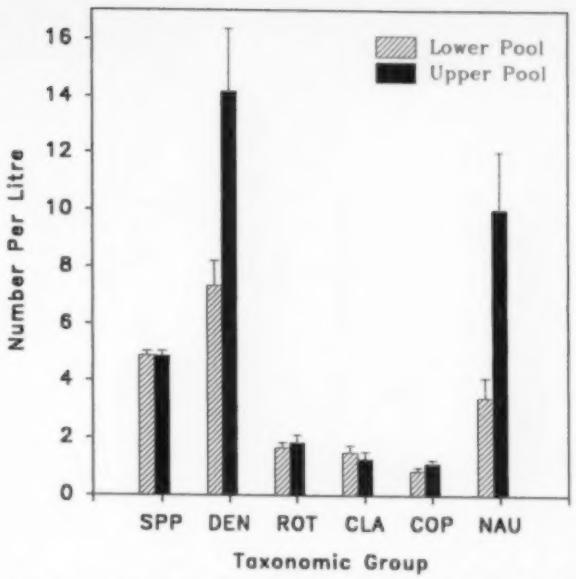

FIG. 7. Mean number of species and individuals (±1 SE) in the lower (hatched bars) and upper regions (solid bars) of a pool.

in the autumn (i.e., late Phase II). Data on density patterns for the most common of 23 species of rotifers, seven species of cladocerans, and various copepods encountered in our riverine samples are given in Table 1.

Although our study was not designed to establish causal relationships between densities of various taxa and abiotic and biotic variables, some possible relationships are suggested by correlation statistics. Species richness of zooplankton was not significantly correlated with water temperature, velocity, or turbidity. In contrast, of the 18 taxonomic density variables examined,[3] 12 were positively correlated with water temperature ($P < 0.01$). Density was correlated with water velocity in 13 cases ($P < 0.05$), all of which were negative except for *Brachionus rubens* ($P < 0.001$) which reached its greatest densities in early Phase II (May) when water velocities were still moderately high. Sixteen density variables were negatively correlated with turbidity ($P < 0.05$).

Our data are generally not useful for suggesting biological interactions among species because all significant comparisons were positive. This probably resulted because maximum densities for all species clustered in Phase II. Nonetheless, it is noteworthy that densities of rotifer species, in contrast with those of cladocerans and copepods, were rarely significantly correlated with other taxa (rotifers with copepods: 21%; with cladocera: 4%; and with other rotifers: 4%). In contrast, copepods were significantly correlated with cladocerans and other copepods 100% of the time, as were cladocerans with each other. However, it may be relevant that rotifer densities dropped dramatically in September when copepod numbers peaked (some copepods prey on rotifers) and rose steeply the following month when copepod densities declined (Fig. 5).

[3]Density variables examined: total zooplankton, rotifers, cladocerans, copepods (without nauplii), and nauplii and densities of *B. longirostris*, *Ceriodaphnia reticulata*, *Daphnia* spp., *Ascomorpha* sp., total *Brachionus*, *B. calyciflorus*, *B. rubens*, *Euchlanis* (probably *E. dilatata*), total *Keratella*, *K. quadrata*, *Polyarthra* spp., Calanoida (mostly *Eurytemora affinis*), and Cyclopoida (mostly *D. thomasi*).

Littoral and Pelagic Zooplankton Assemblages

Species richness values in littoral and pelagic areas of the Ohio River were not significantly different overall or by month (except in September: $F = 4.459$ when $P = 0.0414$); this was true for total zooplankton and for rotifers, cladocerans, and copepods in both the main channel and tributaries. Note that the attached and mostly sedentary species of rotifers and microcrustaceans, which are probably more diverse and dense nearshore, were not sampled in this study. In contrast, average annual densities of total zooplankton were higher nearshore ($F = 6.118$, $P < 0.01$) (Fig. 6), but most of this difference was due to high densities of nauplii in shallow, low-velocity areas during the low-flow periods of summer. However, significant differences between habitats were observed for cladocerans in six months, for copepods in five months, but for rotifers only in September. In 80–100% of the significant comparisons, individual groups were more abundant nearshore. On an annual basis, copepods and cladocerans tended to be more abundant in shallow waters whereas rotifers were usually more numerous in the deeper and faster main channel.

Influence of Low-head Dams

One means of analyzing effects of dams on zooplankton is to compare assemblages in the lower and upper portions of a pool (in this case, above the confluence with a major tributary; see Fig. 7). Plankton assemblages could be different because in comparison with the lower-pool habitat, the upper

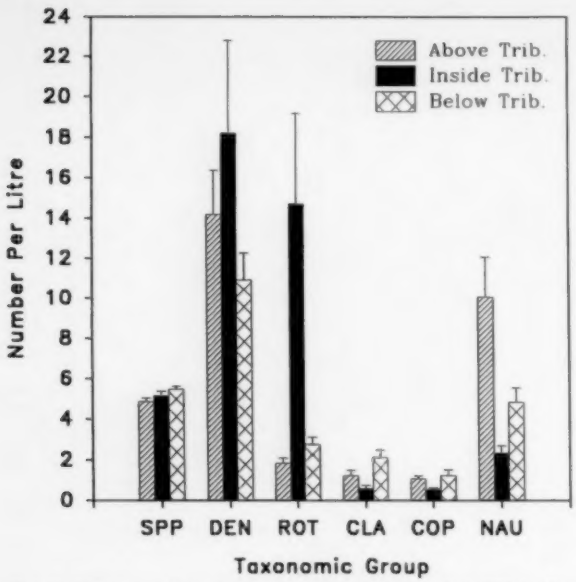

FIG. 8. Mean number of species and individuals (±1 SE) in the tributaries (solid bars) and above (hatched bars) and below (cross-hatched bars) the tributaries in the main channel of the Ohio River.

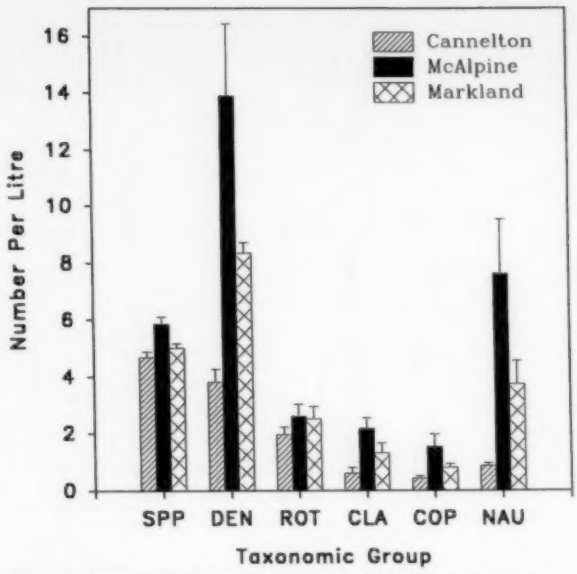

FIG. 9. Mean number of species and individuals (±1 SE) in the Cannelton (hatched bars), McAlpine (solid bars), and Markland pools (cross-hatched bars) of the Ohio River (data drawn only from the four months when all areas were sampled).

pool has higher current velocity, more variable depth, and greater suspended sediment concentration. (J.H. Thorp, personal observation). When data from all three pools were combined, species richness was similar in upper and lower portions of the pools ($F = 0.487$, $P = 0.4857$), but total zooplankton densities were much higher in the upper segment ($F = 8.070$, $P < 0.01$). Differences in total zooplankton density were almost exclusively attributable to nauplii, which were vastly more abundant ($F = 9.168$, $P < 0.01$) in upstream areas of two of the three pools. Juvenile and adult copepods and both rotifers and cladocerans evinced no such pattern as groups. Other than copepod nauplii, only the densities of the rotifers *B. rubens* and *Ascomorpha* sp. were significantly different between upstream and downstream areas of pools; the former was significantly more dense upstream (mean = 0.01/L versus 0.002/L) whereas the latter showed the opposite pattern (0.01 and 0.36/L, respectively).

Therefore, the presence of navigation dams had no detectable effect on either zooplankton species diversity or densities of almost all taxonomic groups. Moreover, significant values which were present were neither consistent among taxonomic groups on a yearly basis nor constant within groups among sample months.

Contrasts with, and Contributions from, Tributaries

Two principal questions were posed in relation to tributaries. First, "Are zooplankton assemblages within tributaries significantly different from those in the Ohio River?" This was examined by comparing zooplankton immediately above a tributary with potamoplankton within a tributary. Second, "Are the diversity and density of the Ohio River zooplankton assemblage significantly influenced by inputs from tributaries?" This was addressed by comparing potamoplankton above and below the tributaries.

Although species richness and total zooplankton density did not differ significantly between tributaries and the main channel of the Ohio River, rotifers were more abundant in tributaries ($F = 8.403$, $P < 0.01$), and the Ohio contained more cladocerans ($F = 4.374$, $P < 0.05$), copepods ($F = 10.947$, $P < 0.001$), and crustacean nauplii ($F = 13.773$, $P < 0.001$) (Fig. 8). The rotifer genera *Keratella* and *Polyarthra* were both more abundant in tributaries ($F = 5.209$ and 5.589, respectively, $P < 0.05$), and *Ascomorpha* sp. was almost significantly different ($F = 3.649$, $P = 0.0568$).

The input of tributaries enhanced diversity ($F = 11.677$, $P < 0.001$) and total density ($F = 7.167$, $P < 0.01$) of potamoplankton in the Ohio River (Fig. 8). Densities in the main channel of the Ohio were greater below than above tributaries for rotifers ($F = 4.152$, $P < 0.05$) and cladocerans ($F = 3.793$, $P < 0.05$) but were lower for copepods ($F = 4.883$, $P < 0.05$), primarily because of decreased densities of nauplii. Numbers of the rotifers *Brachionus* spp. and *Polyarthra* spp. were especially affected. These community shifts occurred without significant changes in physical and chemical conditions downstream of each of the three tributaries. These community patterns were consistent in all tributaries for species richness and densities of rotifers and copepods; but sites above the confluence of the Kentucky River contained greater numbers of total zooplankton, and areas above the mouth of the Miami River had higher densities of cladocerans. In the McAlpine Pool, where all 12 monthly samples were analyzed, diversity and densities tended to be higher below the mouth of the northwest-flowing Kentucky River from July through December but were lower from January through April and mixed in May through June.

Interpool Comparisons

To determine effects of channel/floodplain morphology, we compared average densities and diversities of zooplankton in the lower portion of each pool during the four months

when samples from all pools were analyzed. Total species density was related to pool type ($F = 4.315$, $P < 0.05$), as were densities of adult and juvenile copepods, nauplii, and four species of rotifers; species richness was not significantly influenced by pool type. Although total zooplankton densities were highest in the constricted Markland Pool, this was entirely due to nauplii, which were more than seven times as abundant there than in the other two pools. When these larval copepods were excluded from the analysis, however, the highest densities occurred in the floodplain portion of the Cannelton Pool. Rank order of pools (i.e., 1–3, with 1 = highest density) for densities varied substantially among taxa. Indeed, average ranks among pools were Cannelton (mean = 1.89), McAlpine (1.89) and Markland (2.22) for all significant comparisons, or McAlpine (1.89), Cannelton (2.00), and Markland (2.11) when nauplii were removed. Therefore, clear distinctions among pools were not evident according to channel morphology and floodplain width.

When all sample sites were included in the analysis (rather than just the lower pool), species richness ($F = 9.229$, $P < 0.0001$), total zooplankton density ($F = 9.629$, $P < 0.0001$), and most other taxonomic comparisons were significantly related to pool type (Fig. 9). The average rank orders were 1.50, 2.00, and 2.50 for McAlpine, Markland, and Cannelton pools, respectively.

Discussion

Knowledge of lotic plankton communities is extremely limited in comparison with data available for either lotic benthos or lentic plankton. A contributing factor is the lack of consensus on the nature of potamoplankton assemblages. Do they constitute independent, reproducing communities, or are they merely assortments of transient organisms whose densities are maintained only by constant immigration from backwaters and tributaries? Although production rates have not yet been identified, it is clear from frequent observations of ovigerous individuals that Ohio River zooplankton are a reproducing community (A.R. Black and J.H. Thorp, unpublished data). The lack of consensus on potamoplankton is exacerbated by a common misconception that conclusions drawn from lentic studies can be easily applied to large rivers, negating the need to conduct "superfluous" riverine studies. The spurious nature of this comparison has been admirably demonstrated by Ryder and Pesendorfer (1989). Planktonic communities in navigable rivers probably differ more from lake assemblages than do the more frequently studied reservoir communities because the physical parameters of larger navigable rivers are relatively more disparate. In comparison with lakes and reservoirs, for example, main channels in rivers are well mixed vertically and, therefore, rarely show thermal or chemical stratification. Concentrations of key nutrients (especially N and P) are high in the Ohio River, compared with many lentic systems; more importantly, their seasonal dynamics are apparently regulated by variations in river discharge (i.e., physical processes), rather than by aquatic biota. Advective transport and suspended sediment concentration also have substantial effects on diversity and net secondary production of riverine zooplankton (e.g., Saunders and Lewis 1988b; Pace et al. 1992). Neither factor is consequential in lakes, and except for highly turbid impoundments, both parameters are less significant in reservoirs.

Despite the paucity of studies, several conclusions can be drawn from past research on riverine zooplankton. Density tends to peak in late spring through midsummer (e.g., Neitzel et al. 1982), often with different density modes for rotifers and microcrustaceans. We found similar results for potamoplankton in the Ohio during the period of our study, although peak densities occurred in mid- to late summer. The seasonal timing of the initial rise in densities varied among major taxa, but peak numbers overlapped within a month in the Ohio. Although rotifers are often the numerically dominant group in large rivers, followed in relative importance by copepods and cladocerans (e.g., Shiel 1985; Saunders and Lewis 1988a; Ferrari et al. 1989), this can vary among rivers and seasons (De Ruyter van Steveninck et al. 1990; Pace et al. 1992). During 1991–92 in the Ohio River, the order of numerical abundance averaged over the year (ignoring nauplii) was rotifers (especially *Polyarthra*), cladocerans (predominately *Bosmina*), and copepods (mostly *Eurytemora* and *Diacyclops*); rotifers were nearly twice as abundant as other taxa. *Bosmina* and rotifers are also important in the Hudson River, which like the Ohio is dominated by small zooplankton (Pace et al. 1992). Pace et al. (1992) hypothesized that this size phenomenon could arise because small species should be favored in advective systems, especially turbid rivers with higher concentrations of filamentous and/or toxic algae. The average density of the total zooplankton community was lower in the Ohio than in the tidally influenced Hudson, which fits with the prediction of Pace et al. (1992) that densities should be highest in lakes followed by tidal and then nontidal rivers.

The consensus of opinion dating at least as far back as Hynes (1970) seems to be that plankton assemblages in rivers are physically controlled; while our present data are not at odds with this conclusion, it is noteworthy that biological control mechanisms remain essentially unexplored. Our data for Ohio River zooplankton show a distinct two-phase pattern of species abundance. Phase I (December–April in our sample year) was characterized by high discharge and suspended sediment concentrations, cooler temperatures, mostly diminished nutrient levels, and low zooplankton densities (1.6/L). Phase II (May–November) was characterized by the opposite physical/chemical patterns and relatively high densities (21.6/L; maximum monthly density = 34.6/L). The causal factor or factors for low densities in Phase I are not entirely evident. Densities and reproductive rates of zooplankton in rivers (exclusive of riverine reservoirs) are often not significantly correlated with phytoplankton abundance (Pace et al. 1992) but are closely tied to current velocities and suspended sediment concentrations (e.g., Saunders and Lewis 1988b). While it is true that the low suspended sediment concentrations associated with the slow currents in Phase II should have a positive influence on the ingestion of food particles and body growth of zooplankton (c.f. Kirk and Gilbert 1990), the higher phytoplankton densities and warmer temperatures might make an equal or greater contribution to population size of potamoplankton.

Littoral versus Pelagic Potamoplankton

Habitat characteristics of lakes and rivers (including those with navigation dams) possess significant similarities and differences. Like the littoral zone of lakes, nearshore areas in the Ohio River feature snags (woody debris), vascular macrophytes, and benthic algae which rarely occur in the pelagic zone; they are also more turbulent because of wave

action. Unlike lentic habitats, however, distinctive horizontal or vertical thermal zones are lacking through most or all of the year. Consequently, the littoral zone of a lake typically has a different mixture of species from that present in its pelagic zone. Is the same contrast present between the littoral and pelagic zones of well-mixed rivers?

Our results may suggest that littoral and pelagic areas of rivers differ to a certain degree. Although species richness was similar in shallow (nearshore) and deepwater (mid-channel) areas, average annual densities of total zooplankton were significantly higher nearshore (principally in summer). On an annual basis, copepods and cladocerans tended to be more abundant in the littoral zone whereas nonattached rotifers were usually more dense in deeper and faster main channel waters. These trends may not reflect distinctive niches for zooplankton, however, because the greater density in shoreline areas may be a simple result of lower velocities which tend to retain species and magnify densities. Our sampling methods were not designed to collect benthic cladocerans (e.g., chydorids), copepods, and attached rotifers, and presumably they are much more abundant in the littoral zone. The ecology of benthic rotifers and microcrustaceans in lotic systems is a fertile area for future research.

Influence of Low-head Dams and Tributaries

Ecological perspectives on the effects of dams on lotic ecosystems are based almost entirely on studies of high dams. By integrating the effects of high dams and tributaries on the RCC, Ward and Stanford (1983) developed the serial discontinuity concept. This theory indicates that high dams and large tributaries will cause the downstream biotic community to be "reset" toward an ecosystem state more representative of lower order streams. If the serial discontinuity concept is applicable to rivers with low-head dams, one would expect substantial differences in density, diversity, and/or species composition of potamoplankton in waters above and below a dam or major tributary. Our data suggest, however, that this theory cannot easily be applied to the Ohio River because we found no significant effects of the low-head navigation dams on species diversity or density of zooplankton (with the exception of copepod nauplii).

The influence of tributaries on Ohio River zooplankton appears more substantial and complex than the effect of navigation dams on this community. In their model of the Hudson River zooplankton community, Pace et al. (1992) assumed that tributaries were not a source of the cladoceran *B. longirostris* (the dominant zooplankter in their study) because those streams "... are small, have high current velocities, and are unlikely to support populations of zooplankton" (p. 1065). Our data indicate that a similar assumption would clearly be inappropriate for a model of the Ohio River and its larger tributaries because the Miami, Kentucky, and Salt rivers contribute substantial numbers of zooplankton to the Ohio. Although species richness and total zooplankton density did not differ significantly between tributaries and the main channel of the Ohio River during our study, rotifers were significantly more abundant in tributaries, and the Ohio contained significantly more cladocerans and copepods. The input of major tributaries significantly enhanced diversity and total density of potamoplankton in the Ohio River. Densities in the main channel of the Ohio were greater below than above tributaries for rotifers and cladocerans but were lower for copepods, primarily because of decreased densities of nauplii. Even though tributaries significantly influenced zooplankton assemblages in the Ohio River, evidence of a tributary-induced reset phenomenon is lacking over the 361-km stretch of the river studied here. While this may reflect unique characteristics of this very large river, we suspect that a primary reason for nonconformance with this theory is that the serial discontinuity concept was based principally on the behavior of benthic insects, whose food resources change dramatically along the river continuum.

Interpool Comparisons

Recent studies of large river ecosystems (Junk et al. 1989; Sedell et al. 1989) predict that rivers with constricted channels should more nearly conform to the RCC (Vannote et al. 1980) than lotic systems with wide floodplains. A comparison of zooplankton assemblages in the lower portions of two constricted-channel pools and one floodplain pool of the Ohio River produced no evidence supporting this important theory. In contrast, when all samples within a pool were averaged (i.e., both upper and lower reaches), differences among floodplain and constricted-channel pools were highly significant. These dramatic contrasts in results suggest that assemblages in the upper portion of the pools are being affected by different environmental variables than those in lower areas of the three pools. A factor likely to be highly influential is the upstream proximity of large urban areas with point-source and non-point-source inputs of pollutants. Specifically, the upper portion of the more floodplain Cannelton Pool is immediately below Louisville, and the upper reach of the Markland Pool is downstream from Cincinnati. In contrast, the upper McAlpine Pool is in a more rural area.

In North America, freshwater zooplankton biologists work almost exclusively in lentic habitats, while stream zoologists rarely study large rivers or species other than vertebrates or benthic invertebrates (e.g., insects, snails, and crayfish). Consequently, the fundamental biological knowledge of large rivers in general and potamoplankton in particular is abysmal, making it especially difficult now to tackle ecological questions as complex as those addressed in other habitats or with other species. It is evident from our Ohio River study, however, that physical factors (current velocity, turbidity, and temperature) strongly influence potamoplankton. In this navigable river with low-head dams, zooplankton assemblages appear to vary between some habitats (littoral versus pelagic) but not others (upper versus lower pool and floodplain versus constricted-channel pools). Zooplankton clearly reproduce within the Ohio, but immigrants from tributaries also may contribute significantly to the community of the main channel. Future studies are needed to determine whether internal production alone can sustain potamoplankton and whether biological interactions are important in regulating these species. Use of novel experimental techniques, such as drifting, Nitex mesh pelagic enclosures, or "potamocorrals" (Thorp 1992b; J.H. Thorp et al., unpublished data), may be necessary to understand more fully the ecology of potamoplankton in large rivers.

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Effect of Changes in Streamflow on the Microhabitat Use and Movements of Sympatric Juvenile Coho Salmon (*Oncorhynchus kisutch*) and Chinook Salmon (*O. tshawytscha*) in a Natural Stream

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The microhabitats at positions selected by juvenile coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*) following a change in streamflow differed from microhabitats occupied at normal streamflows. At drought streamflow (37% mean seasonal streamflow (MSF)), juvenile coho salmon selected slower, darker, and higher sites above the streambed ($P < 0.05$) than sites selected at normal (75% MSF) or flood (159% MSF) flows. Juvenile chinook salmon microhabitat use changed similarly with changes in streamflow, but the differences were not significant. Up to one fifth of the fish chose positions with faster water velocities than those available either 30 cm above or 30 cm lateral to them. These fish chose positions inconsistent with the hypothesis of optimal position selection based on maximizing net energy gain. On average, fish moved 6.8 m following a change in streamflow. Juvenile coho salmon generally moved upstream in response to decreasing streamflows and downstream in response to increasing streamflows. Juvenile chinook salmon tended to move offshore and downstream in response to all streamflow changes. These results show that juvenile coho and chinook salmon will move to find suitable microhabitat following a change in streamflow and that the microhabitats are not the same at all streamflows.

Les microhabitats aux emplacements choisis par les jeunes saumons coho (*Oncorhynchus kisutch*) et quinnat (*O. tshawytscha*) après un changement de débit diffèrent des microhabitats occupés lorsque le débit est normal. Au débit de sécheresse (37 % du débit saisonnier moyen (DSM)), le jeune saumon coho choisit des sites plus sombres, plus élevés par rapport au lit du cours d'eau et où l'écoulement est plus lent ($P < 0,05$) qu'au débit normal (75 % DSM) ou au débit de crue (159 % DSM). L'utilisation du microhabitat par le jeune saumon quinnat varie également en fonction du débit, mais les différences observées sont non significatives. Jusqu'à un cinquième des poissons choisissent des emplacements où la vitesse d'écoulement de l'eau est plus élevée que ceux disponibles à 30 cm d'eux, en hauteur ou latéralement. Le choix des emplacements par ces poissons est contraire à l'hypothèse de la sélection optimale de l'emplacement basée sur l'optimisation du gain d'énergie nette. En moyenne, les poissons se déplacent de 6,8 m consécutivement à un changement de débit. Le jeune saumon coho se déplace en général vers l'amont en réponse à une diminution du débit, et vers l'aval en réponse à une augmentation du débit. Le jeune saumon quinnat a tendance à quitter le rivage et à se déplacer vers l'aval en réponse aux changements de débit de tout type. Ces résultats indiquent que les jeunes saumons coho et quinnat se déplaceront pour trouver un microhabitat adéquat consécutivement à un changement de débit, et que les microhabitats ne sont pas les mêmes quel que soit le débit.

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Changes in river discharge (referred to as "streamflow" herein) associated with flow regulation can have negative effects on fish and wildlife resources (Petts 1984; Burt and Mundie 1986). As a result, numerous "instream flow" assessment methods have been developed for use throughout North America to evaluate environmental impacts and minimize losses of fish habitat (Reiser et al. 1989). Some of these assessment methods incorporate attributes of the habitat used by fish (e.g., the Instream Flow Incremental Methodology (IFIM), Bovee, 1982). The requirement of specific levels of habitat attributes for these methods has stimulated investigations into the basis on which fish choose positions in streams (e.g., Shirvell 1990). Fausch (1984), Fausch and White (1986), Hughes and Dill (1990), and others have suggested that juvenile salmonids select stream positions that allow optimal foraging. Specifically, juvenile salmonids in streams are hypothesized to select positions in slow current close to faster water with an abundant food supply. Such a choice would allow the fish to maximize its net energy gain by reducing its energy expenditure. Positions with these characteristics can be considered for feeding to be "optimal positions" where the greater the water velocity gradient next to the position, the greater the potential for net energy gain. When streamflows change, the locations that allow maximum net energy gain will also change because of the shift in patterns of water velocity (Bravender

and Shirvell 1989). If the hypothesis of optimal positions is correct, when stream locations that allow maximum net energy gain change because of changes in streamflow, fish should redistribute themselves and take up new optimal positions. Although instream flow assessment methods such as IFIM assume that fish have the same habitat preferences following a change in streamflow, no formal test of the correspondence between optimal locations and fish positions following a change in streamflow has been conducted.

The purposes of this paper were to (1) compare water depths and velocities used by the same juvenile coho (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*) before and after a change in streamflow to determine whether their microhabitat use remained consistent, (2) compare the positions used before and after a change in streamflow to determine whether the fish moved to find new locations with suitable conditions, and (3) examine the specific water velocity gradients around positions the fish chose at three different streamflows to determine whether they selected positions in slow water close to faster water consistent with optimal stream position theory.

Methods

Study Area

The study was conducted in Kloiya Creek, a small coastal stream 12 km southeast of Prince Rupert, British Columbia. Kloiya Creek has a series of reservoirs and a short mainstem emptying directly into the Pacific Ocean. There is a dam with a fishway 3.2 km upstream from the ocean. The study area was a 235-m-long section of stream consisting of a series of five alternating pools and riffles approximately 800 m downstream of the dam. The pools were consecutive but in two groups: pools 1 and 2 formed the upstream group and were separated from pools 3, 4, and 5 by 100 m of stream. At the average annual streamflow the five pools averaged 47 m long, 21 m wide, 52 cm maximum depth, and averaged 18 $cm \cdot s^{-1}$. They had predominantly cobble substrate with gravel and sand along their margins. A complete physical description of the study area is given in Bravender and Shirvell (1989).

Kloiya Creek has annual spawning escapements of about 200 chinook salmon, 700 coho salmon, and populations of at least three other anadromous species (Shirvell 1990). Although the dam has a fishway, all the chinook salmon and about 30% of the coho salmon spawn downstream of the dam in two localized areas with spawning gravels. The study area used for this research was within the upper spawning area. After emerging from the gravel, the juvenile coho and chinook salmon remain and grow in the study pools for periods ranging from a few days to several months before they emigrate to sea.

Streamflow Manipulation

To investigate the consistency of water depths and velocities occupied by juvenile salmon at different streamflows, the flow in Kloiya Creek was experimentally altered. By storing or releasing water from the reservoir upstream of the dam, the flow in the study area could be regulated between 0.5 and 8.0 $m^3 \cdot s^{-1}$. Flow was manipulated at the dam to create alternating low, medium, and high levels in the study area.

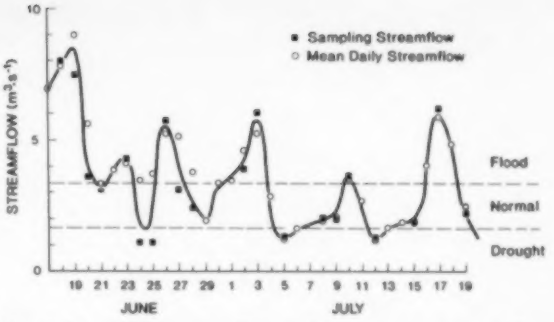

FIG. 1. Mean daily streamflow and instantaneous streamflows when fish habitat measurements were taken in Kloiya Creek, B.C., from June 17 to July 19, 1985 (redrawn from Shirvell 1990). Streamflow classifications were as follows: drought, <1.65 $m^3 \cdot s^{-1}$; normal, 1.65–3.35 $m^3 \cdot s^{-1}$; flood >3.35 $m^3 \cdot s^{-1}$.

The low flows averaged 1.25 $m^3 \cdot s^{-1}$ (range 1.17–1.64 $m^3 \cdot s^{-1}$), medium flows averaged 2.56 $m^3 \cdot s^{-1}$ (range 1.65–3.35 $m^3 \cdot s^{-1}$), and high flows averaged 5.42 $m^3 \cdot s^{-1}$ (range 3.36–8.02 $m^3 \cdot s^{-1}$). The experiment was conducted from June 18 to July 19, 1985. The mean seasonal streamflow (MSS) during June and July each year from 1975 to 1984 was 3.41 $m^3 \cdot s^{-1}$. For the experiment, I chose low, medium, and high streamflows that approximated drought (37% MSS), normal (75% MSS), and flood conditions (159% MSS) relative to the normal June and July flows. Over the 32-d study period, five floods, four normal flows, and three droughts were simulated (Fig. 1).

Measurement of Microhabitat Characteristics at Fish Positions

Typically, habitat used by a fish population is determined from measurements of conditions at positions occupied by a sample of different fish collected on different days and assumed to be representative of an entire population on each sample date. Because water depths and velocities used by fish are related to their size, samples composed of different fish could result in different mean depths and velocities due to random sample variation. To prevent habitat variation caused as an artifact from measuring conditions occupied by different fish on different sampling dates, in this study I measured the habitat conditions occupied by the same fish at different streamflows on different days.

Individual juvenile coho and chinook salmon were located in the study area by direct underwater observation. Twenty surveys lasting 7 h each were used to locate the fish and measure habitat attributes at their position. A diver with snorkel quietly entered one of the pools at its downstream riffle and carefully moved upstream until a juvenile salmon maintaining position in the stream was observed. The diver noted the location and position of the fish in relation to the substrate and then captured the fish alive in a small, handheld purse seine (Morantz et al. 1987).

Upon capture of the fish the following habitat attributes were measured at each fish position: (1) water depth, (2) vertical distance of the fish above the streambed, (3) water velocity at the fish's head (the focal point velocity), (4) light intensity at the focal point, (5) light intensity at the water surface, and (6) dominant substrate particle size. The location of the fish's position within the pool was determined by triangula-

TABLE 1. Description of juvenile salmon tagged and relocated following a change in streamflow in Kloiya Creek, B.C., 1985.

Species	Number tagged	Mean length, mm (SD)	Mean weight, g (SD)	Number never relocated	Number of times relocated									
					1	2	3	4	5	6	7	8	9	10
Coho	40	55.4 (7.1)	1.9 (1.1)	17	6	6	3	1	2	1	2	0	2	0
Chinook	37	53.8 (3.6)	1.7 (0.4)	27	4	3	1	1	1	0	0	0	0	0
Total	77			44	10	9	4	2	3	1	2	0	2	0

tion using measurements from the position to permanent metal pins marking the ends of transects used for hydraulic measurements (see Bravender and Shirvell 1989). The positions were later marked on scale maps of the study pools.

To test whether fish in the study pools chose positions consistent with the hypothesis of optimal position selection (Hughes and Dill 1990; Fausch 1984), (7) water velocity 30 cm lateral to the fish's position on the faster side of the position and (8) water velocity 30 cm above the fish's position were also measured. This distance was chosen because Wankowski (1981) found that the distance juvenile Atlantic salmon (*Salmo salar*) would move to capture prey varied seasonally from 1.9 to 9.9 body lengths. The juvenile coho and chinook salmon in this study were approximately 55 mm in length (Table 1); therefore, 30 cm represented 5.5 body lengths. All water velocity measurements were taken with an Ott current meter using a 3.0-cm propeller following the recommendations of Morantz et al. (1986). Light intensity was measured with a Licor integrating quantum photometer (model LI-212SB). The dominant particle size of the substrate at any location was visually determined and then a representative particle was measured and coded using the modified Wentworth classification (see Orth 1983).

Once the habitat measurements were taken, the fish was anaesthetized, weighed (nearest 0.1 g), and measured (fork length in millimetres). Each fish was then tagged with an individually colour-coded aluminum staple. These tags were attached to the fish by pinching the ends of the staple into the back of the fish on either side of the dorsal fin. When attached, the tag hung behind the dorsal fin and rested on the fish's back between the dorsal and adipose fins. Each tag was marked with a unique two- or three-colour code of enamel paint on the top of the staple. The colour code could be read underwater by the diver without capturing the fish. Following measurements and tagging, the fish was allowed to recover and was then released near its capture location.

On subsequent observations of tagged fish, the tag code was noted and all the habitat variables described above were remeasured at the position the fish was occupying. The distance and direction that tagged fish moved between subsequent observations were measured from their locations marked on scale maps of the study pools. The direction in which a fish moved between two observations was coded according to the numbers on a clock, where 12 = upstream, 6 = downstream, 3 = inshore, and 9 = offshore. Because fish may be influenced by the nearest shore when searching for new positions following a streamflow change, the coding for inshore and offshore was reversed for fish occupying locations on the right-hand side (facing downstream) of the thalweg. This transformation standardized code 3 to always represent inshore (generally shallower) and 9 to always represent offshore (generally deeper) for analysis of the direction the fish moved in regardless of which side of the thalweg the fish was initially on before the streamflow changed (see Fig. 4 for an example).

The change in streamflow between two consecutive observations of the same fish was calculated and paired with the distance and direction the fish had moved between the two observations. All observations were taken between 08:30 and 17:00 from June 18 to July 19, 1985.

Statistical Analysis

To test the variability of microhabitat conditions selected by the fish between different streamflows, the means of the habitat attributes at the positions selected for each of the three streamflows were analyzed for significant differences with Duncan's multiple range test (DMRT) at $P = 0.05$. Differences in habitat conditions selected by tagged versus untagged fish, differences between habitat conditions selected by coho and chinook salmon, and differences between water velocities at the fish's position and water velocities lateral or vertical to the fish's position were analyzed using t-tests or paired t-tests at $P = 0.05$. The existence of any relationship between the distance fish moved as a result of a streamflow change and the magnitude of the streamflow change was examined using linear regression. The directions fish moved in response to a streamflow change were plotted graphically, where the direction the fish most frequently moved in was normalized to 1. All other directions the fish moved toward were plotted as proportions relative to the most frequently used direction. The transformation of the direction of movement data to normalized proportions was done to allow comparisons between different changes in streamflow or comparisons between the coho and chinook when the sample sizes were unequal. All statistical analyses were conducted using SAS systems software (SAS Institute Inc. 1985).

Results

Effect of Tagging on Microhabitat Choice

To investigate whether tagging the fish affected their choice of microhabitat, the conditions at the locations where the fish were captured before they were tagged were compared with the conditions at the locations where the fish were located on their first observation following tagging. None of the habitat attributes investigated was significantly

TABLE 2. Microhabitat conditions occupied by juvenile coho salmon at three streamflows in Kloiya Creek, B.C.

Mean of habitat variable	Streamflow ($m^3 \cdot s^{-1}$)			All three streamflows
	1.25	2.42	5.38	
Depth (cm)	39.1 [29][a] (10.6)	37.9 [43] (9.6)	35.6 [49] (9.0)	37.3 [121] (9.6)
Distance above bottom (cm)	12.6 [29] (8.3)	11.2 [43] (9.4)	8.1[b] [49] (4.3)	10.3 [121] (7.6)
Focal point velocity ($cm \cdot s^{-1}$)	11.3 [29] (7.7)	16.4 [43] (7.4)	18.7 [49] (6.2)	16.1 [121] (7.5)
Lateral velocity ($cm \cdot s^{-1}$)	19.0 [29] (9.6)	25.4 [43] (8.5)	26.8 [49] (9.6)	24.5 [121] (9.6)
Vertical velocity ($cm \cdot s^{-1}$)	24.9 [29] (11.8)	34.8 [43] (14.5)	34.6 [49] (17.2)	32.3 [121] (15.6)
Light at fish's position ($lx \cdot 10^3$)	7.0 [28] (4.4)	13.8 [23] (9.0)	13.6 [37] (14.5)	11.6 [88] (11.1)
Light at surface ($lx \cdot 10^3$)	12.3 [28] (8.8)	26.0 [23] (19.7)	22.3 [37] (24.7)	20.1 [88] (20.1)
Substrate (cm)[c] (label)	6.4 (gravel)	15.7 (cobble)	3.3 (gravel)	6.4–25.0 (cobble)
Distance from shore (m)	4.6 [28] (2.6)	4.9 [40] (2.2)	3.9 [40] (2.2)	4.5 [108] (2.3)

[a]Sample size in square brackets; SD in parentheses.
[b]Underlined values are significantly different from the means at the other streamflows.
[c]Mode of frequency histogram.

different between the two locations for either species (coho $N = 23$, chinook, $N = 10$, $P > 0.05$, paired t-test) except for water velocities 30 cm above the fish's position ($P < 0.01$, paired t-test) and light intensities at the water surface above the fish positions ($P < 0.05$, paired t-test). These results show no difference in the microhabitat conditions at the fish's focal point before or after tagging, but some differences existed in the conditions above the fish after tagging. These results suggest that once juvenile coho and chinook salmon were tagged, they may have selected locations underlying faster water than before they were tagged. I speculate that this may be a fright reaction to the tagging procedure. Nevertheless, other than the fish possibly selecting positions under faster water, these results show that the tags appeared to have had no effect on the fish's swimming ability and subsequent choice of water velocities at the fish's position. It further suggests that habitat conditions used by the tagged fish were valid samples of habitat conditions that would be chosen by the population at different streamflows.

Juvenile Coho and Chinook Salmon Microhabitat Differences

A total of 40 juvenile coho and 37 juvenile chinook salmon were tagged (Table 1). Of these, tagged coho salmon were observed after a streamflow change on 66 occasions; some individuals were observed after as many as nine streamflow changes. Tagged chinook salmon were observed after a streamflow change on 22 occasions; some individuals were observed after as many as five streamflow changes. The average length of time between consecutive observations of the same fish was 5.1 d (SD = 3.8 d; min. = 1, max. = 22). There were substantially fewer juvenile chinook salmon relocated than juvenile coho salmon. This was because the chinook salmon were emigrating to the ocean during the study period. The coho will remain in freshwater for a year before emigrating. There was no significant difference between the length or weight of either species ($P = 0.24$, t-test; Table 1).

Comparisons between species showed that juvenile coho and chinook salmon chose different microhabitats for each streamflow tested in Kloiya Creek. Although the water depth ($P = 0.37$), water velocity at the fish's position ($P = 0.45$), water velocity 30 cm lateral to the fish's position ($P = 0.71$), and light intensity at the fish's position ($P = 0.17$) were not significantly different between locations chosen by either coho or chinook salmon, the water velocity 30 cm above the fish's position, distance above the streambed, and distance from shore were significantly different ($P < 0.05$) at different streamflows. Juvenile chinook salmon chose locations that were significantly farther from shore ($P = 0.04$ or less), closer to the streambed ($P = 0.0017$), and underneath significantly faster currents ($P = 0.0013$) at all streamflows.

Consistency of Habitat Use between Different Streamflows

Juvenile coho and chinook salmon each occupied different microhabitats at different streamflows. For coho salmon, the values of water velocity at their position, the water velocity 30 cm lateral to their position, and the water velocity 30 cm above their position were significantly lower at drought streamflow than at normal or flood streamflow, while they were higher for light intensity ($P < 0.05$, DMRT; Table 2). These attributes were not different between the positions coho salmon chose at normal and flood streamflows. The water depth, substrate size, and distance from shore at the positions chosen by coho salmon were consistent for all three streamflows ($P > 0.05$, DMRT; Table 2).

For juvenile chinook salmon, the water velocity 30 cm above their position was significantly lower at drought streamflow than at either normal or flood streamflow ($P < 0.05$,

TABLE 3. Microhabitat conditions occupied by juvenile chinook salmon at three streamflows in Kloiya Creek, B.C.

Mean of habitat variable	Streamflow ($m^3 \cdot s^{-1}$) 1.25	2.42	5.38	All three streamflows
Depth (cm)	37.0 [9][a] (10.7)	34.4 [19] (10.8)	36.1 [31] (12.7)	35.7 [59] (11.7)
Distance above bottom (cm)	9.4[b] [9] (4.2)	5.3 [19] (2.1)	7.3 [31] (4.0)	7.0 [59] (3.7)
Focal point velocity ($cm \cdot s^{-1}$)	12.1 [9] (7.7)	17.2 [19] (7.3)	18.7 [31] (10.3)	17.2 [59] (9.2)
Lateral velocity ($cm \cdot s^{-1}$)	17.7 [9] (12.1)	25.8 [19] (8.7)	24.4 [31] (11.8)	23.8 [59] (11.1)
Vertical velocity ($cm \cdot s^{-1}$)	25.0 [9] (17.4)	43.9 [19] (12.9)	43.2 [31] (15.0)	40.6 [59] (16.0)
Light at fish's position ($lx \cdot 10^3$)	8.8 [9] (3.7)	17.0 [16] (5.1)	14.7 [28] (15.8)	14.4 [53] (12.1)
Light at surface ($lx \cdot 10^3$)	15.3 [9] (6.0)	30.0 [16] (9.9)	25.7 [28] (28.0)	25.2 [53] (21.6)
Substrate (cm)[c] (label)	3.3 (gravel)	15.7 (cobble)	15.7 (cobble)	6.4–25.0 (cobble)
Distance from shore (m)	7.1 [7] (3.0)	6.8 [15] (1.7)	5.9 [24] (2.8)	6.3 [46] (2.5)

[a]Sample size in square brackets; SD in parentheses.
[b]Underlined values are significantly different from the means at the other streamflows.
[c]Mode of frequency histogram.

TABLE 4. Correlation between streamflow, fish's distance above the streambed, and water velocity at the fish's position for juvenile coho and chinook salmon in Kloiya Creek, B.C.

Species	Streamflow (1.25 $m^3 \cdot s^{-1}$) (2.42 $m^3 \cdot s^{-1}$) (5.38 $m^3 \cdot s^{-1}$)	Variables (streamflow) (height above bottom) (water velocity)	Pearson correlation coefficient (R)	Probability (P)	Number of observations (N)
Coho, chinook	All	Flow, height	−0.25	0.0008	180
Coho, chinook	All	Flow, velocity	0.25	0.0009	180
Coho, chinook	2.42	Flow, height	−0.29	0.0244	62
Coho	All	Flow, height	−0.27	0.0032	121
Coho	All	Flow, velocity	0.32	0.0004	43
Coho	2.42	Flow, height	−0.30	0.0508	9
Chinook	1.25	Height, velocity	−0.62	0.0757	19
Chinook	2.42	Height, velocity	−0.40	0.0940	32
Chinook	5.38	Height, velocity	0.38	0.0319	32

NOTE: 27 other correlation analyses (8 distance above bottom with streamflow, 10 water velocity at fish position with streamflow, and 9 water velocity at fish position with distance above bottom) were conducted that had $P > 0.10$.

DMRT; Table 3). The mean water velocity at the position chosen by the chinook salmon, the mean water velocity lateral to their position, the mean light intensity above their position, the modal substrate size, and their mean distance from shore were different, but the difference was not statistically significant at $P < 0.05$. This may be due to the small sample size.

The size of the difference between the means of these variables and the size of their variances at different streamflows were the same for the chinook salmon as they were for the coho salmon. The only difference between the coho and chinook salmon data was that fewer chinook salmon positions ($N = 22$) were measured than coho salmon positions ($N = 66$). Therefore, had the sample size of the chinook data been larger, the differences in these habitat attributes used by juvenile chinook salmon at the different streamflows may have been significant as well.

Juvenile coho and chinook salmon changed their position in the water column and the water velocities they occupied in response to changes in streamflow. The height above the streambed occupied by coho during the flood streamflows was significantly less than what they used at normal or drought streamflow ($P < 0.05$, DMRT; Table 2). For juvenile chinook salmon, their distance above the streambed was significantly higher at drought streamflow than at either normal or flood streamflow ($P < 0.05$, DMRT; Table 3). The distance above the streambed occupied by coho and chinook salmon was negatively correlated with streamflow, but the water velocity at the positions they occupied was positively correlated with streamflow (Table 4). At drought

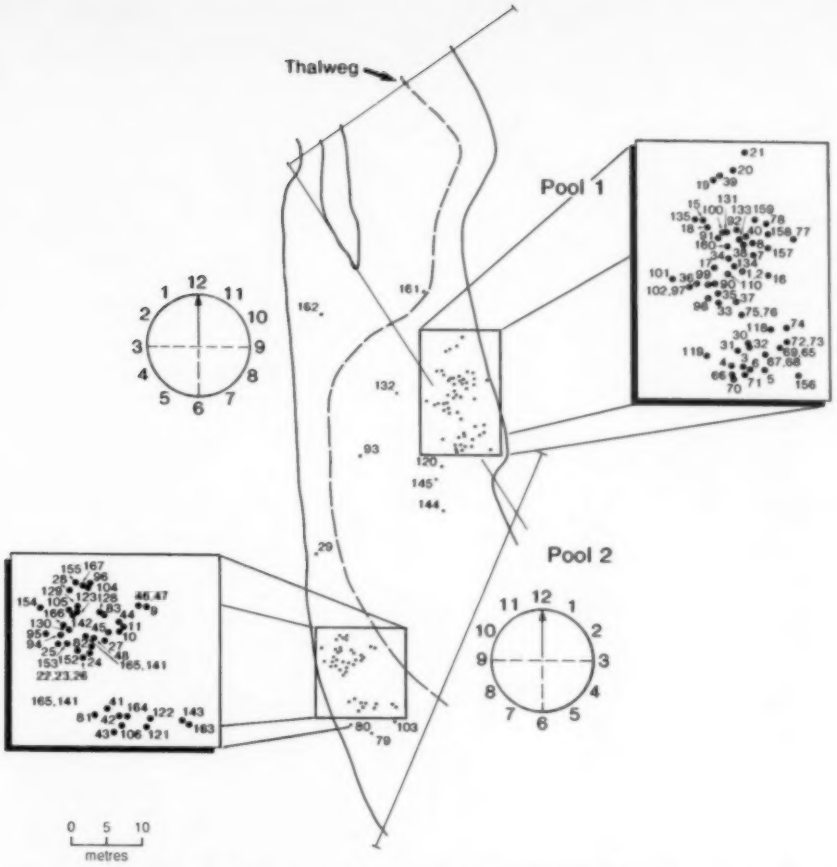

FIG. 2. Distribution of positions used by juvenile coho and chinook salmon in pools 1 and 2 of Kloiya Creek, B.C., at 1.25, 2.42, and 5.38 $m^3 \cdot s^{-1}$. The direction the fish moved in response to a streamflow change was coded according to clock positions (12 = upstream). Note the reversed clock numbers on the right side of the river (looking downstream). Numbers of individual fish positions are from Bravender and Shirvell (1990).

and normal streamflows, the water velocity at the position that juvenile chinook occupied was negatively correlated (Pearson correlation) with their distance above the streambed, but at flood streamflows, it was positively correlated.

Water Velocity Gradients around Fish Positions

The focal point velocity at positions chosen by both species was significantly slower than the water velocity both adjacent to and above the positions ($P < 0.06$, t-tests). Additionally, for both coho and chinook salmon, the mean water velocity 30 cm above the fish was significantly faster than the mean water velocity 30 cm lateral to the fish at all streamflows (coho $P = 0.001$, chinook $P = 0.001$).

The analyses above, because they use the mean of water velocities at many fish positions, do not detect inconsistencies in the patterns of water velocity gradients around individual fish. The positions selected by all juvenile coho and chinook salmon were not always slower than surrounding water velocities. Eighteen percent (21 of 121) of positions occupied by coho salmon had focal point water velocities that were faster than water velocities 30 cm lateral to the faster side of the fish (mean = 4.4 $cm \cdot s^{-1}$ faster, max. = 9.0 $cm \cdot s^{-1}$ faster) and 10% (13 of 121) had focal point water velocities that were faster than water velocities 30 cm above the fish (mean = 4.8 $cm \cdot s^{-1}$ faster, max. 12.0 $cm \cdot s^{-1}$ faster (Bravender and Shirvell 1990)). Two positions occupied by coho salmon had focal point water velocities that were faster than velocities both lateral and vertical to the fish's focal point. For positions occupied by juvenile chinook salmon, 19% (12 of 59) had velocities faster than velocities 30 cm lateral to the fish (mean = 3.4 $cm \cdot s^{-1}$ faster, max. = 10.0 $cm \cdot s^{-1}$ faster) and 2% (1 of 59) had velocities faster than velocities 30 cm above the fish.

Fish Movements

Juvenile coho and chinook salmon usually selected locations near the edge of the stream and distributed themselves in concentrated clumps. An example of their distribution in pools 1 and 2 is shown in Fig. 2. When streamflow changed, the fish moved to new locations. The average distance moved by both species for all streamflow changes was 7.2 m (N = 97, SD = 7.9 m). Juvenile chinook salmon generally moved

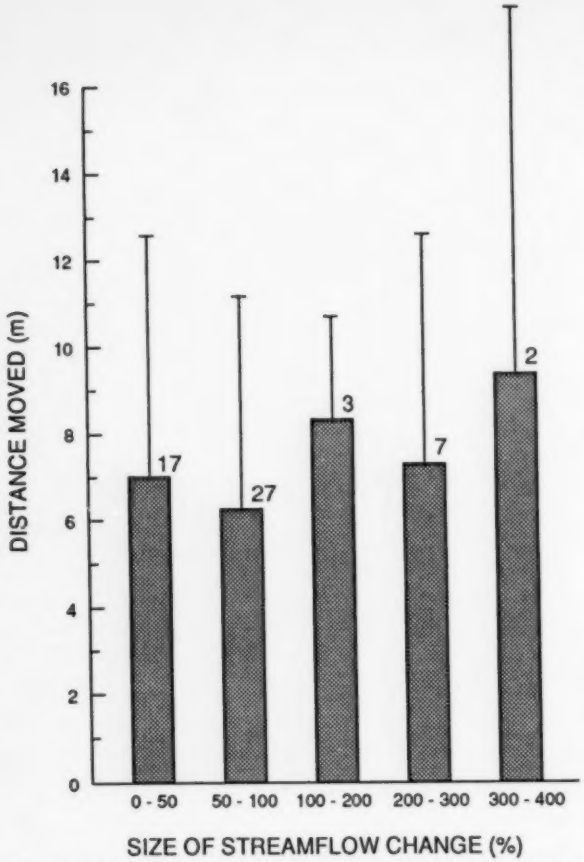

FIG. 3. Distance moved by juvenile coho and chinook salmon in Kloiya Creek, B.C., in response to changes in streamflow: Distance moved (m) = 5.91 + 0.57(% change in streamflow ($m^3 \cdot s^{-1}$)) ($R^2 = 0.55$, P that $b \neq 0$ is 0.15). The size of the streamflow change is expressed as a percentage of the initial streamflow. Error bars show 1 SD of the mean, and the numbers at the top of the bars are the sample sizes.

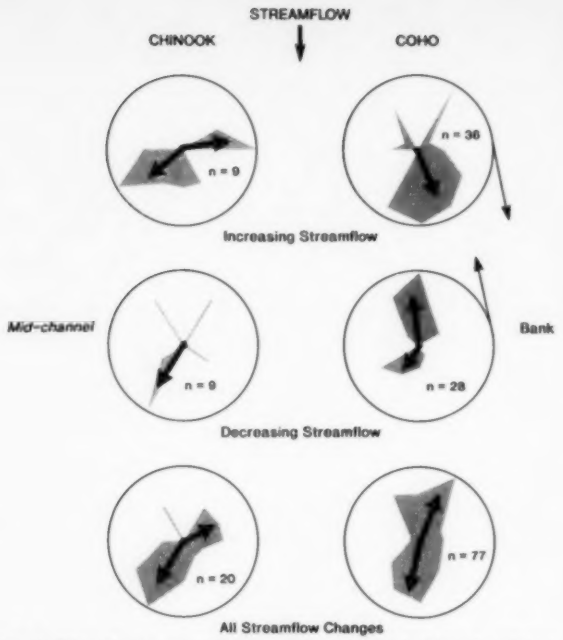

FIG. 4. Directions moved by juvenile coho and chinook salmon in Kloiya Creek, B.C., following changes in streamflow. The frequency of all directions in which the fish moved was normalized and shown in proportion to the most frequently used direction. "All streamflow changes" includes movements following increases and decreases in streamflow plus movements for intervals where the streamflow on the relocation date was the same as the streamflow at the previous observation. Arrows on the edges of the circles for coho salmon indicate the shift in the water's edge with increases or decreases in streamflow.

farther than coho salmon following a change in streamflow (mean distance of 8.6 versus 6.9 m, respectively); however, the difference was not significant ($P = 0.37$, t-test). The movements of each fish between successive observations are shown in Bravender and Shirvell (1990).

The mean distance the fish moved following a streamflow change was positively correlated with the size of the streamflow change, but only weakly (Fig. 3). Most fish remained in the pool where they were originally captured; only two coho and one chinook salmon moved to a different pool following a streamflow change. Two of these fish moved to a pool upstream. The mean distance moved by all three fish exceeded 50 m. Seventeen of the coho salmon that were tagged (43%) and 27 of the chinook salmon that were tagged (73%) were never observed again. These fish apparently left the pool where they were originally captured following a streamflow change. While it is unknown where these fish moved, it is probable that the chinook salmon, at least, went downstream into the estuary.

Juvenile coho and chinook salmon generally moved in different directions following a streamflow change. Juvenile coho salmon moved predominantly downstream and parallel to the water's edge with increasing streamflow and upstream parallel to the water's edge with decreasing streamflow (Fig. 4). In contrast, juvenile chinook salmon moved predominantly offshore and downstream regardless of the direction of the streamflow change, but especially with decreasing streamflow. With increasing streamflow, juvenile chinook salmon tended to move perpendicularly inshore.

The direction moved by juvenile coho salmon appeared to be unrelated to the size of the streamflow change. Movements following both an increase and a decrease in streamflow greater than 60% from the previous streamflow were no different from movements made following an increase or a decrease in streamflow less than 60% from the previous streamflow. There were insufficient data to analyze the chinook salmon movements for any relationship with the size of the streamflow change.

The mean distance of the positions occupied by both the coho and chinook salmon relative to the water's edge was the same at all streamflows (4.5 and 6.3 m, respectively; Tables 1 and 2). However, because the shoreline shifted an average 2.0 m when streamflow increased from drought to normal streamflow and an additional 2.9 m when streamflow increased from normal to flood streamflow, the juvenile coho and chinook salmon must have also moved laterally by the same amount for their mean distance from shore to remain unchanged.

Discussion

Juvenile coho and chinook salmon occupied different microhabitats in Kloiya Creek. When they were the same size, the water depths and velocities they selected were similar (Tables 1 and 2), but the locations juvenile chinook salmon occupied were usually farther out from shore in brighter light and over larger substrate. Additionally, juvenile chinook salmon stayed closer to the streambed and were always beneath much faster currents than were juvenile coho salmon. Despite these differences, both species occupied the same general areas of the stream (e.g., Fig. 2). In Kloiya Creek, coho and chinook salmon shared the available habitat in the rearing areas in subtle ways. Lister and Genoe (1970) found similar habitat segregation between juvenile coho and chinook salmon during their first 3 mo following emergence in the Big Qualicum River, B.C., a comparable coastal stream. It appears that each species has different specific habitat preferences that allow them to intermingle in heterogeneous rearing areas without competing for the same microhabitats.

When streamflow changed, juvenile coho and chinook salmon moved to new locations characterized by their specific microhabitat preferences. The average distance moved by the fish exceeded the average distance that the isopleths of habitat conditions used by the fish at the former streamflow shifted (the fish moved an average of 6.8 m, while the depth and velocity contours shifted laterally an average of 2.0–2.9 m). While the fish demonstrated that they could discriminate between and select new positions with habitat conditions similar to their previous ones, they did not appear to be efficient at occupying the closest possible site with suitable conditions. I speculate that this inefficiency in occupying the closest suitable position may have been caused by the randomness of searching combined with behavioural interactions with other fish.

Movements made by juvenile salmon in response to rapid increases in streamflow in Kloiya Creek were voluntary and not due to their inability to swim against the increased current. For coho salmon, many of their movements after a streamflow change were upstream. Even when movements were downstream, such as those made predominantly by the chinook salmon, the distances were not great, generally less than 10 m. Furthermore, of all the fish relocated after a streamflow increase, only three left the pool where they were first observed. Both the coho and chinook salmon did not use the deepest water available to them, even at the highest streamflows. When streamflow increased to flood, neither species used positions much deeper than 40 cm (Tables 1 and 2) even though 40% of the study area had greater depths available (Bravender and Shirvell 1989). Heggenes (1988) suggested that, based on data from Tschaplinski and Hartman (1983) and Heggenes and Traaen (1988), young fish might be "washed-out" from streams by abrupt increases in stream discharge. While there was no evidence of this in Kloiya Creek, the coho and chinook salmon in this study were much larger than the fish studied by Heggenes and Traaen (1988) (54 versus 30 mm). The size of the streamflow increase used in this study was also much less than used by Heggenes (1988) (2 times increase versus 4–100 times increase, respectively). All movements and subsequent selection of habitat by the juvenile coho and chinook salmon in this study appeared to have been by choice.

Juvenile salmon in Kloiya Creek avoided displacement by high streamflows by moving to new locations with lower water velocities. When streamflows increased, both the juvenile coho and chinook salmon initially moved toward the stream bed (Tables 1 and 2) and then laterally (Fig. 4) to position themselves in suitable velocities. However, the juvenile chinook salmon moved greater distances on average to locate new "suitable" locations than did juvenile coho salmon (8.6 versus 6.8 m). A possible explanation for this might be that the centre of the stream was physically less heterogeneous than the edges of the stream. Because juvenile chinook salmon usually occupied the middle of the stream while the coho usually stayed along shore (see Shirvell 1990) the distances between physical structures that caused suitable reductions in the current (boulders or large pieces of wood?) may have been greater in the middle of the stream than they were near the shore.

Although the habitats used by juvenile coho and chinook salmon at the three streamflows were similar, they were not identical. The means of several of the habitat attributes at positions the fish occupied were significantly different between the low and the normal and high streamflows. This suggests that the fish may have had different habitat preferences at each of the streamflow levels assuming that the forces of social interaction remained constant between streamflows. It is not certain that these differences in habitat use were strictly due to different habitat preferences, however, because habitat use can be affected by the amount and kind of habitat that is available (Baldridge and Amos 1982). Nevertheless, the area of suitable habitat available in Kloiya Creek was so large that it seems reasonable that a surplus of appropriate habitat was available at all streamflows (Bravender and Shirvell 1989). If so, then the different habitat used at the three streamflows would reflect different habitat preferences.

Position selection by juvenile salmonids in streams has been suggested to be due to the fish's attempt to acquire food efficiently, i.e., they are hypothesized to select "optimal stream positions" in moderate currents near fast water where, by minimizing their energy expenditure near abundant food sources, they maximize their net energy gain (Fausch 1984). An alternative hypothesis is that the fish select positions with combinations of preferred levels of water velocity, depth, and substrate, where their probability of occurrence is 1.0 (Bovee 1982). If optimal position selection is correct, then selection of positions in streams would not be based on a preferred water velocity per se (the IFIM hypothesis), but would be based on locations with velocity gradients, where the stronger the gradient, the greater the potential for net energy gain. Fausch (1984) showed that juvenile coho salmon occupied positions with the highest potential for net energy gain available to them. However, Hughes and Dill (1990) showed that for solitary Arctic grayling (*Thymallus arcticus*), the position that allowed the maximum net energy gain was not necessarily in slow current next to strong velocity gradients because the Arctic grayling had to be close enough to the prey to detect it so that they could react.

Juvenile coho and chinook salmon usually selected positions in Kloiya Creek where water velocity increased in any direction away from them. However, because of the effect of streambed friction on waterflow in streams, it is normal that there is a positive water velocity gradient at increasing dis-

tance away from the streambed. Consequently, it is usual that any position near the streambed or banks would have the water velocity gradient of optimal position theory. However, coho and chinook salmon selected positions 18 and 19% of the time, respectively, in water that was faster than water velocities 30 cm lateral to them, and coho salmon selected positions 10% of the time in the water column that were faster than water velocities 30 cm above them. Similar position choice has been found for juvenile chinook salmon in the Nechako River (Nechako River Project 1987). There, 26% of the juvenile chinook salmon (78 of 304) selected positions that were faster than water velocities 30 cm lateral to them and 19% (58 of 299) selected positions in the water column faster than those 30 cm above them. Thus the juvenile salmon in Kloiya Creek selected positions that were inconsistent with the optimal stream position hypothesis. The juvenile coho salmon also selected different levels of focal water velocity, focal light intensity, and height above the bottom of Kloiya Creek at different streamflows. Therefore, they also selected positions that were inconsistent with the constant habitat preference hypothesis of IFIM.

While the lack of a positive velocity gradient around all positions selected by salmonids in streams does not refute an energetic basis for position selection some of the time, it suggests that the fish consider other needs besides feeding. As the predominant motivation changes from feeding to some other need, the microhabitat conditions required by the fish may also change, thus accounting for the different habitat use at different streamflows that occurred in Kloiya Creek. I propose that fish have different habitat preferences for different activities. As an example, when the primary purpose is feeding, fish would select optimal positions along velocity gradients, as found by Fausch (1984). When the primary purpose is avoidance of predators, fish would select optimal positions along light gradients, as found by Heggenes et al. (1993). Shirvell and Dungey (1983) found significant differences between the microhabitats used by brown trout (*Salmo trutta*) for feeding and spawning, while Shirvell (1990) found that different levels of water velocity were selected by steelhead (*Oncorhynchus mykiss*) parr depending on whether they were sheltering from current velocity (feeding?) or hiding from predators. Thus, fish have activity-specific habitat preferences. Because the activity a fish is engaged in can be affected by streamflow, the assumption that habitat use by juvenile coho and chinook salmon is constant for all streamflows is false, and this has important significance for models that attempt to predict the effects of streamflow change on fish habitat.

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Distribution and Movements of Belugas, *Delphinapterus leucas*, in the Canadian High Arctic

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Belugas, *Delphinapterus leucas*, move into the coastal waters of Somerset Island as soon as the annual land-fast ice breaks up in late June – early July. Six bays or inlets which receive the outflow of rivers are the major areas of summer aggregation. Belugas captured and equipped with satellite-linked UHF transmitters in Cunningham Inlet (Barrow Strait), Elwin Bay (Prince Regent Inlet), and Creswell Bay moved west into Peel Sound where they frequented two other estuaries. Rapid and directed movement out of Peel Sound occurred in late August. All of the animals which transmitted locations into September or October moved to eastern Devon Island and Jones Sound. The longest period of transmission was 75 d, lasting until mid-October. Belugas tagged in three different locations around Somerset Island in the summers of 1988–93 showed a well-defined and consistent pattern of behaviour. Aerial surveys done during this period confirm that the vast majority of belugas in this region are involved in these seasonal movements.

Les bélugas, *Delphinapterus leucas*, entrent dans les eaux littorales de l'île Somerset dès la débâcle annuelle de la banquise côtière, entre la fin de juin et le début de juillet. Six baies ou bras de mer dans lesquels se déversent les cours d'eau constituent les principales zones de regroupement estival. Les bélugas capturés et équipés d'émetteurs UHF à liaison satellitaire dans l'inlet Cunningham (détroit de Barrow), la baie Elwin (inlet Prince Regent) et la baie Creswell, se sont déplacés vers l'ouest jusqu'au détroit de Peel, où ils ont fréquenté deux autres estuaires. À la fin du mois d'août, on a observé un mouvement rapide et dirigé de sortie du détroit de Peel. Tous les animaux dont la localisation a été faite par leur émetteur en septembre ou octobre se sont déplacés vers l'est jusqu'à l'île Devon et le détroit de Jones. La période d'émission la plus longue a été de 75 jours et a duré jusqu'à la mi-octobre. Les bélugas marqués à trois différents endroits autour de l'île Somerset au cours des étés 1988 à 1993 ont montré un profil de comportement bien défini. Les levés aériens effectués durant cette période confirment que la vaste majorité des bélugas dans cette région participe à ce mouvement saisonnier.

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Most belugas, *Delphinapterus leucas*, occupying the eastern Canadian High Arctic during the ice-free months of July and August are found in the coastal shelf habitats around Somerset Island, N.W.T. This region, including Barrow Strait and parts of Lancaster Sound, is usually covered by land-fast annual sea ice from October to late June. Aerial surveys of belugas in autumn (Finley and Johnson 1977) have shown a movement out of Barrow Strait and eastward through Lancaster Sound. Belugas have been reported to overwinter in the large North Water polynya of Baffin Bay (Finley and Renaud 1980), but it is believed that most of the High Arctic stock spends the winter in the pack ice off the west coast of Greenland between Disko Bay and approximately 63°N (Vibe 1950; Kapel 1977). Recent surveys in west Greenland (Heide-Jorgensen 1992) indicate that the area south of 65°N is no longer important as winter habitat for belugas, possibly because of excessively high catches there in drive-net fisheries between 1917 and 1930. The main wintering concentrations are presently found between 67°N and 69°N, and not more than 80 km from the west Greenland coast.

The little evidence to date points to a shared common stock of belugas between the eastern Canadian High Arctic and Greenland. The continuing high annual harvests in west Greenland and small harvests in Canadian waters impose an annual estimated removal of 1200 animals per year (IWC 1991). This population has been estimated to comprise between 6300 and 18 600 animals, uncorrected for those below the water surface when the survey aircraft passed overhead (Smith et al. 1985). Even if the upper bound of the estimate is inflated by a factor of 1.42 to compensate for these missed whales (Martin and Smith 1992), the total harvest probably far exceeds the sustainable yield for any odontocete whale population (Sergeant 1981; Burns and Seaman 1988; Doidge 1990). Added to the harvest are the frequent ice entrapments of belugas which, combined with opportunistic hunts at these "sassat" (Porsild 1918; Freeman 1968; Kapel 1977; Heide-Jorgensen 1994), result in significant added mortality. As recently as February 1990, a "sassat" occurred in Disko Bay where an estimated 500 belugas were killed by Inuit hunters. Comparisons of relative abundance of belugas counted in Greenland waters during the

TABLE 1. Details of transmitter pack deployment on belugas at three sites on Somerset Island, N.W.T., Canada. Whales were captured and released at the freshwater outlets of Cunningham Inlet (CI) (74°05′N, 93°49′W), Elwin Bay (EB) (73°33′N, 90°57′W), and Creswell Bay (CB) (72°43′N, 93°20′W) between 1988 and 1993.

Release date	Local time	Place	Tag No.	Transmitter type	Details of animal			Tag longevity (d)
					Length (m)	Sex	Age	
22 July 1988	13:58	CI	8753	Location	3.35	F	Adult	10.6
27 July 1989	17:33	CI	5802	Location	3.20	F	Adult	7.0
30 July	18:37	CI	5800	Location	2.50	F	Subadult	0.01
17 July 1990	12:07	CI	5801	Location and depth	2.90	F	Subadult	11
17 July	17:22	CI	8751	Location	3.05	F	Adult	37.6
17 July	17:22	CI	8750	Location	3.24	F	Subadult	29.6
18 July	15:36	CI	5803	Location and depth	4.04	F	Adult	20.5
23 July	13:00	CI	5806	Location and depth	4.16	F	Adult	44.9
21 July 1991	12:24	CI	5801	Location and depth	3.81	F	Adult	27.6
27 July	21:45	CI	5804	Location and depth	2.64	F	Adult	0.05
28 July	21:25	CI	5805	Location and depth	3.40	F	Subadult	24.6
31 July	16:00	CI	8750	Location and depth	3.88	F	Adult	47.2
1 Aug.	12:50	CI	8757	Location and depth	3.71	F	Adult	2.5
26 July 1992	11:06	EB	8752	Location and depth	3.88	F	Adult	75.0
23 July	15:00	EB	8753	Location and depth	4.64	M	Adult	72.7
23 July	14:10	EB	8754	Location and depth	4.18	F	Adult	69.8
25 July	20:40	EB	8755	Location and depth	4.31	F	Adult	64.7
21 July	13:30	EB	8756	Location and depth	4.50	M	Adult	0.1
13 Aug. 1993	02:45	CB	8750	Location and depth	3.80	F	Adult	58.9
14 Aug.	14:00	CB	8752	Location and depth	3.66	F	Adult	0.1
15 Aug.	18:15	CB	8753	Location and depth	3.80	F	Adult	20.9
15 Aug.	15:30	CB	8754	Location and depth	3.40	F	Subadult	0.1
14 Aug.	12:50	CB	8755	Location and depth	3.88	F	Adult	46.5

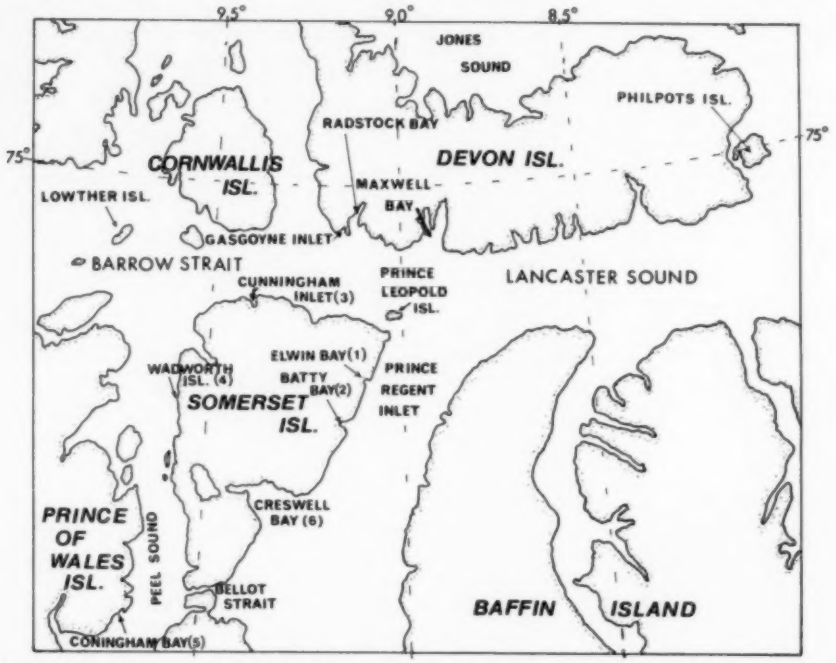

FIG. 1. Map of Somerset Island and adjacent open-water areas occupied by belugas, with the five (1–5) main areas of estuarine aggregation.

winters of 1981 and 1982 with surveys in 1990 and 1991 seem to indicate a significant decline in numbers consistent with the expected effect of the high continuing annual harvests (Heide-Jorgensen et al. 1992).

In light of the need for a reassessment of the size and status of this stock, we review our knowledge of the seasonal distribution and numbers of belugas found in their summering areas in Canadian waters and present direct evidence on

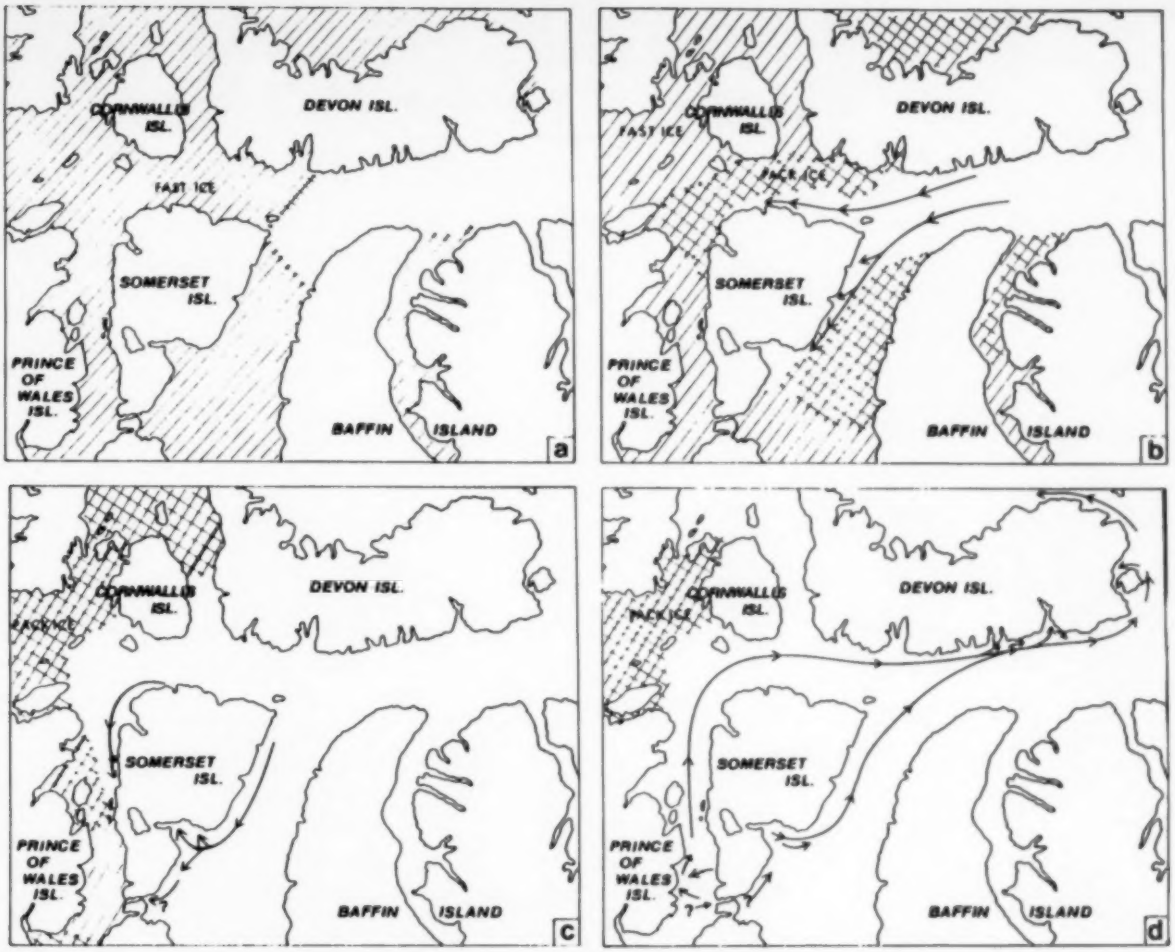

FIG. 2. (a) Extent of land-fast ice during a typical winter in Barrow Strait and Lancaster Sound. (b) Initial ice breakup from June to early July. Arrows indicate direction of travel of belugas in early summer. (c) Further clearing of ice in late July in Barrow Strait and Prince Regent Inlet. Arrows indicate direction of travel by belugas in late July – August. (d) Late August – September distribution and movement (arrows) of belugas from Peel Sound.

seasonal movements and migration routes from our studies of belugas equipped with satellite-linked UHF transmitters.

Methods

The coastal areas of Somerset Island including Barrow Strait, Prince Regent Inlet south to Creswell Bay, and Peel Sound south to Bellot Strait were surveyed opportunistically by Bell 206 helicopter or Twin Otter aircraft during July and early August from 1980 to 1992. Flight levels of 340 and 170 m asl were selected, the lower level when taking vertical photographs through a hole in the chin bubble of the Bell 206B helicopter using a Hasselblad 70-mm-format camera equipped with an 80-mm lens.

From 1988 to 1993, 23 belugas, 16 adult and five subadult females and two adult males, were captured by driving them into the shallows and then restraining them using hoop nets, fitted with satellite-linked UHF transmitters and immediately released (Martin and Smith 1992) (Table 1). Thirteen were captured at Cunningham Inlet, five at Elwin Bay, and five in the Creswell Bay area (Table 1; Fig. 1). The transmitter specifications, package design, and attachment procedures have been described in Martin and Smith (1992) and Martin et al. (1993). Details of service Argos, the satellite data reception system used, and estimates of data accuracy are given in Fancy et al. (1988) and CLS/Service Argos (1989).

Each of our transmitters was identified by a unique number and transmitted up to 256 data bits derived from onboard sensors. The early packages transmitted signals used to determine location only whereas the most recent also relayed both detailed and summary data on dive profiles and swimming velocity (Table 1).

Average speeds of travel were only estimated between pairs of Argos-derived high-quality locations (location of quality = 2 or 3). To further reduce error, we restricted our data set to locations separated by time intervals of ≥5 h.

Data were analysed using the Proc. Sort procedures of SAS (SAS Institute Inc. 1989). Grid location analysis was done by assigning numbers to geographic grid squares of 20′ latitude by 1° longitude to cover the entire study area.

TABLE 2. Numbers and locations of belugas observed during reconnaissance surveys in the waters adjacent to Somerset Island.

Period	Barrow Strait (90°–95°20′W)				Prince Regent Inlet (72°40′–74°N)			Creswell Bay (Fury Point to Creswell River)	Peel Sound (72°50′–74°N)	Coningham Bay	Ice conditions
	Cunningham Inlet	Somerset northcoast	Devon coast	Flow edge	Elwin Bay	Batty Bay	Open coast				
8 Aug. 1980	800	?	549	N/A	?	?	?	1995	Frozen		
14 July 1982	94–192	31	?	N/A	170	0	+	?	Frozen		
13 July 1983	0	0	0	3000	?	?	?	?	?		Floe edge far east
24 July 1984	Frozen	Frozen	Frozen	2000	Frozen	Frozen	Frozen	Frozen	Frozen		
1 JulyFrozen	Frozen	+	660	Frozen	Frozen	Frozen	Frozen	Frozen			Floe edge
3 Aug.	300	20	?	N/A	0	300	0	2000+	?		Floe edge
12 Aug.	0	0	0	N/A	?	?	?	?	?		
20 July 1987	Frozen	500+	Few	N/A	75	?	?	?	?		
31 July 1988	2000	1158	?	N/A	550	81	118	1426	Frozen		
2 Aug.	1000								2000		
6 Aug.	400–500	?	?	N/A	?	?	?	?	50		Estimated 8–9/10
8 Aug.	?	?	0	N/A	0	?	92	599	30		ice Peel
9 Aug.	400–500	?	?	N/A	?	?	?	?			Sound
								?			8/10 ice north of
26 July 1989	200	59	?	N/A	800	600	123	4100	6 (frozen)		Birmingham
3 Aug.	0	?	?	N/A	0	296	38	3300	59		Bay
4 Aug.	300	?	?	N/A	150	200	300	(Some? No counts)	?		
29 July 1991	80	75	?	N/A	125	441	212	4298	0		
20 July 1992	?	?	?	N/A	600	?	?	?	?		Peel Sound
5–8/10											
27 July2000	?	?	N/A	?	?	?	?	?			ice
29 July2500	?	?	N/A	?	?	?	?	?			
30 July1800	0	?	N/A	?	0	34	458+	Frozen			
1 Aug.	600	124	?	N/A	60	51	133	1588			Peel Sound
2 Aug.	300	?	?	N/A	?	?	?	?	?		8/10 ice
6 Aug.	50	0	?	N/A	?	?	?	?	1316		
7 Aug.	?	?	1329	N/A	?	?	?	?	?		
18 July 1993	900	49	?	N/A	363	227	7	0 (9/10 ice)	?	?	
20 July1600–1700	?	?	N/A	?	?	?	?	?	?		
4 Aug.	300	?	?	N/A	264	100	147	1922	1 (6/10 ice)	?	
6 Aug.	?	?	0	N/A	172	603	357	2067	107	488	
15 Aug.	?	?	0	N/A	0	85	78	339	?	?	

Unless otherwise stated, the following results derive from a combination of aerial survey, radio-tracking, and ground-based observational data.

Results

Beluga Movements in Relation to Ice Conditions and Pattern of Breakup

Access by whales to their summering areas around Somerset Island is limited by the land-fast ice which forms during the winter and spring months in Lancaster Sound, Prince Regent Inlet, Barrow Strait, and Peel Sound. The timing of breakup depends heavily on the winter location of the fast-ice edge which can vary greatly from year to year (Marko 1982; Gorman 1988). In an average year the ice edge forms across Barrow Strait from Prince Leopold Island to Maxwell Bay on Devon Island (Fig. 2a).

In June, as shore leads develop, both southward into Prince Regent Inlet along the east coast of Somerset Island and westward into Barrow Strait along the north coast, whales begin moving to their areas of summer aggregation (Fig. 2b). A consistent feature of this breakup pattern is a series of large leads, apparently caused by coastal upwelling, running parallel to the northern Somerset Island coastline from Prince Leopold Island (Prisenberg and Bennett 1987). By late July the fast-ice edge has usually receded to Lowther Island, but ice cover in Barrow Strait is quite variable as broken ice shifts position and ice empties out of adjoining channels.

In Cunningham Inlet, a main area of beluga concentration in July (Smith et al. 1992), fast ice begins to melt near the delta as a result of river outflow in the latter part of June. Tidal currents are believed to initiate breakup at the northern entrance to the inlet, followed by melting along the western shore. The last ice to remain in the inlet is usually found in the northeast and southeast portions of the bay. These areas are normally ice free by mid-July.

During the same period, Prince Regent Inlet has cleared of ice, allowing whales access to Elwin Bay, Batty Bay, and eventually Creswell Bay, all areas of fresh warm water influx where the nearshore ice has melted (Fig. 2c).

Peel Sound on the west coast of Somerset Island retains its ice into August. A shore lead develops along the Somerset coast, allowing belugas to penetrate southwards into Peel Sound, where they concentrate along the floe edge and in leads as the ice breaks up. Belugas concentrate at the entrance to a small river north of Wadworth Island. Later, when the ice has broken up completely in Peel Sound, large numbers of belugas concentrate in the freshwater outflow of Coningham Bay, situated on the coast of Prince of Wales Island in Franklin Strait (Fig. 2d).

Number of Belugas in Areas Adjacent to Somerset Island

From coastal aerial surveys in areas adjacent to Somerset Island and land-based counts made mainly at Cunningham Inlet (Smith et al. 1992), we have compiled a general picture of the summer distribution and autumnal dispersion of belugas (Table 2). When coastal surveys were flown from late July into August (e.g., 1988 and 1992) including the main areas of aggregation such as Cunningham Inlet, Creswell Bay, and Peel Sound, the numbers of belugas and timing and pattern of dispersion were quite similar between years.

Belugas in the Barrow Strait area initially concentrate in Cunningham Inlet, where numbers build rapidly from mid-July to the end of the month. During that period, numbers of belugas frequenting Elwin Bay and Batty Bay, in Prince Regent Inlet, also increase. Our general impression from those surveys was that belugas in Prince Regent Inlet moved down to Creswell Bay where numbers peak in the early part of August; some 4000+ belugas are found in the shallow bays receiving the outflow of freshwater from the different arms of the Creswell River.

By the end of the first week of August, there is a major shift in the distribution of the belugas centred around Cunningham Inlet in Barrow Strait. The ice in Peel Sound begins to break up rapidly and the whales from Barrow Strait push southward into the shore leads and fractured ice. Often the floe edge establishes itself briefly in an east–west direction across Peel Sound from Somerset Island side near Wadworth Island – Birmingham Bay to Back Bay on Prince of Wales Island. There, thousands of belugas aggregate, with as many narwhals, *Monodon monoceros*, and numerous harp seals, *Phoca groenlandica*. Belugas push into the mouth of an unnamed river (73°30′N, 95°42′W) north of Wadworth Island where they exhibit the same rubbing behaviour (Smith et al. 1992) as seen earlier in Cunningham Inlet. When the ice finally permits passage, belugas push further south into Franklin Strait and usually remain in the area of Coningham Bay with its freshwater outflow and the adjacent deep marine trench. Rarely, belugas push still further south, where they are occasionally hunted by the Inuit from Spence Bay (69°30′N).

We know less of the dispersion of the large concentration of belugas found in Creswell Bay during August. During this study, belugas were sighted south of Creswell Bay as far as Bellot Strait, but while significant numbers of narwhals were seen swimming west against the strong currents of Bellot Strait, we have never documented belugas using this route to reach the large aggregation of this species in the Coningham Bay area. Significant numbers of belugas were, however, seen in mid-August along the southern coast of Devon Island, often in Gascoyne Inlet and Radstock Bay. Belugas are possibly attracted to these areas because of the occasional nearshore presence of large schools of Arctic cod, *Boreogradus saida* (Welch et al. 1992).

Effects of Capture on Belugas in Estuaries

We tagged belugas at three sites, Cunningham Inlet, Elwin Bay, and Creswell Bay, which were very different from each other with respect to the disturbance which was imposed on the whales during the capture process. Cunningham Inlet and Elwin Bay were both defined and enclosed bays whereas the captures at Creswell were spread out along an area of coastline. The larger of the two bays, Cunningham Inlet, allowed us to isolate our capture effort and minimize disturbance to other whales. This was achieved by keeping our activities behind an acoustic barrier, a sandspit on the south-west side of the Bay. At Elwin Bay, it was necessary to move our boat across the whole Bay to capture the whales in the river channels. Because of the large area in which we operated in Creswell Bay, we could not measure the effect of our captures on the whales in the area.

At Cunningham, we caused the Inlet to be vacated completely after 13 of 21 capture attempts (62%) whereas at Elwin, four of four attempts emptied the Bay completely.

TABLE 3. Number of attempted captures, frequency of complete disturbance to the estuary (all whales departed), and minimum, maximum, and average time for the first belugas to return to Cunningham Inlet and Elwin Bay.

	No. of capture attempts	No. of times Inlet vacated	Time to return (h)		
			Min.	Max.	Avg.
Cunningham Inlet					
1988	5	4 (80%)	11	21	17.6
1989	6	6 (100%)	6	27	15
1990	3	0 (0%)	5	21	12
1991	7	3 (43%)	11.5	29	17
Elwin Bay					
1992	4	4	11.5	29	17

TABLE 4. Number of tagged belugas which remained in the capture areas after release or which left and later returned.

	Total tags deployed and functional	No. of belugas staying or returning to estuary	No. of belugas which did not leave	Time to first return (h)		
				Min.	Max.	Avg.
Cunningham Inlet						
1988	4[a]	2	1	12		
1989	1	0	0			
1990	5	3	1	183[b]		
1991	4	2	1	25		
Elwin Bay						
1992	4	4	0	7	143	41.5

[a]Three conventional VHF and one PTT.
[b]Stayed together while travelling; possible mother with older juvenile offspring.

In both areas, belugas returned to the Bay within 12–17 h (Table 3). Of 17 tagged belugas, 11 (65%) returned to the estuary after capture and three belugas did not leave the estuary (Cunningham Inlet) for a period of hours or days after capture. Of those whales which left immediately after capture and returned, the time lapse ranged from 7 to 143 h (Table 4).

Utilization of Estuaries and Offshore Areas by Belugas

We have identified six principal estuarine areas used by belugas on the coasts of Somerset and Prince of Wales islands (Fig. 1). In the three years of study at Cunningham Inlet, when transmitters functioned for sufficient periods of time, none of the tagged whales moved east toward Prince Regent Inlet. The 1990 tag information, which ended by 16 August, indicated a movement into Peel Sound, while the 1991, 1992, and 1993 studies reinforced this picture with more details on the use of two estuarine habitats, the small unnamed river emptying into Peel Sound at Birmingham Bay and the river emptying into Coningham Bay on the coast of Prince of Wales Island.

In 1992, when the belugas were all caught at Elwin Bay and transmissions lasted into late September, we gained a more detailed picture of large-scale movements and use of estuaries. Whales released from Elwin Bay used Batty Bay, but did not travel south to join the large numbers of belugas in Creswell Bay. Instead the general movement was to the west into Barrow Strait, with three of the five belugas venturing briefly into Cunningham Inlet. The movement appears to have been mainly directed toward Peel Sound, where all four belugas with still-functioning transmitters congregated in the small estuary north of Wadworth Island (73°30′N, 95°42′W) and eventually penetrated south to Coningham Bay.

Of the three functional tags applied in 1993 at Creswell Bay, only one whale ventured into Peel Sound and Franklin Strait. None of the three spent much time in estuaries, probably because of the late date of their captures. The only other estuaries frequented by two of those three animals were those of Batty and Elwin bays in Prince Regent Inlet.

The highest frequency of transmissions was received from the locations of Cunningham Inlet and Elwin Bay in July and the small estuary north of Wadworth Island and Cunningham Inlet in August. Coningham Bay was most important as an aggregation area in August 1991, when ice conditions allowed belugas to penetrate into the area as early as 6 August. In 1992 when the ice was much heavier, the small river estuary north of Wadworth Island became the most important area of aggregation in Peel Sound. One whale tagged at Elwin Bay on 23 July reached this area on 6 August. In 1993, ice blocked access to this estuary, but allowed belugas to pass south along the west side of Peel Sound. By September, all belugas had ceased using estuaries.

The proportion of time spent in estuaries by belugas in July and August varied from 0.005 to 0.12. Of the five tags which were applied in mid-July and transmitted until the end of August, the mean proportion of time spent in estuaries was 0.082 (Table 5). It is obvious that belugas tagged late in the season, as in 1993, frequent estuaries much less.

Belugas also used the waters close to estuaries more frequently than other locations. Of the five whales whose transmissions were received from July to at least the end of August the total proportion of their time spent in the estuary or in the offshore area of the grid rectangle which included the estuary varied from 0.29 to 0.38 (Table 6).

TABLE 5. Estuarine utilization by individual beluga whales tagged at Cunningham Inlet, Elwin Bay, and Creswell Bay. Other estuaries occupied include Batty Bay, north of Wadworth Island, and Coningham Bay (see Fig. 1).

Date tagged	Date ended	Tag No. (see Table 1)	Estuaries occupied (Fig. 1)	Total hours tagged, July and Aug.	Proportion of total hours (July–Aug.) in estuaries
Cunningham, 1990					
15 July	26 July	5801	(3)	263:35	0.0485
16 July	27 July	5803	(3)	259:38	0.0486
23 July	21 Aug.	5806	(3) (4)	703:17	0.005
15 July	16 Aug.	8750	(3)	761:57	0.0510
15 July	6 Aug.	8751	(3)	521:27	0.0353
Cunningham, 1991					
21 July	18 Aug.	5801	(3) (5)	661:36	0.0486
29 July	22 Aug.	5805	(3) (5)	575:00	0.053
31 July	13 Sept.	8750	(3) (4) (5)	716	0.0744
1 Aug.	4 Aug.	8757	(3)	33.23	0.0352
Elwin, 1992					
27 July	17 Sept.	8752	(1) (2) (3) (4)	853	0.0314
24 July	4 Oct.	8753	(1) (2) (3) (4)	936	0.1039
24 July	26 Sept.	8754	(1) (2) (4)	920	0.1230
27 July	28 Sept.	8755	(1) (4) (5)	859	0.0796
Creswell, 1993					
13 Aug.	11 Oct.	8750		444	0
15 Aug.	5 Sept.	8753	(6)	361.50	0.018
14 Aug.	28 Sept.	8755	(6) (1) (2)	390.90	0.045

Of the five belugas tagged in July and which transmitted data into at least mid-September, one was tagged at Cunningham Inlet and the rest at Elwin Bay. Peel Sound was by far the most heavily used of the major areas around Somerset Island by all those animals, ranging in total proportion of time tagged from 0.19 to 0.33 in the offshore areas not including time in the estuaries.

The offshore waters of Barrow Strait and Prince Regent Inlet were little used by the whales during the whole period. Lancaster Sound became important again during autumnal eastward migrations (Table 7).

Timing of Major Movements

All of the belugas tagged at either Cunningham Inlet or Elwin Bay from 1990 to 1992 ($n = 15$) moved west into Peel Sound. Only one of three tagged in Creswell Bay in 1993 moved into Peel Sound. In 1990, when animals were tagged as early as 15 July, the earliest arrival in Peel Sound was on 24 July. The latest arrival in Peel Sound was a whale tagged in Elwin Bay on 26 July arriving on 7 August (Table 8).

Once in Peel Sound, belugas congregated at the floe edge, which crossed to Prince of Wales Island at the latitude of the river estuary north of Wadworth Island. Of 11 belugas with tags transmitting into at least mid-August, eight eventually moved south into the Coningham Bay area, arriving as early as 29 July in 1991 and as late as 28 August in 1993.

Of the six whales transmitting data into September, departure dates from Peel Sound occurred between 28 August and 7 September. The animals spent little time in Barrow Strait, moving directly east into Lancaster Sound between 30 August and 15 September (Table 8).

Speed of Travel and Individual Long-range Movements

Travel speed calculated indirectly from location data cannot yield much detail about maximum or minimum swimming capabilities. Our conservative estimates of average travel speed over large time intervals where sufficient sample sizes are available ($N \geq 30$) lie in the range of 1.1–1.85 km/h (Table 9).

During the period of eastern migration from Peel Sound through Barrow Strait and into Lancaster Sound, there is an apparent fast directed movement which brings belugas along the southern coast of Devon Island and eventually into the area of Philpots Island (74°55′N, 79°40′W) and southern Jones Sound in late September. In 1991, whale No. 8750 moved through Barrow Strait covering 298 km in 85 h (1.6 km/h). On 7 and 8 September, it apparently increased its speed to move 132 km in 22 h (5.99 km/h) to longitude 80°W. In 1992, speeds of 2.11, 3.54, and 3.8– 4.9 km/h were estimated during similar movements for three whales over distances of 167–522 km. Caution should be used in evaluating all these estimates because of the potential error from poor location qualities and the small number of samples. More accurate speed estimates can only be made in the future from direct measurements taken with onboard velocity meters.

In 1990, four of the five belugas tagged at Cunningham Inlet were released on 15 or 16 July; one other was released on 23 July. All of the early tagged whales moved into Barrow Strait and three returned one or more times to Cunningham Inlet. All animals moved into Peel Sound by 26 July. There appeared to be a general movement of Cunningham Inlet belugas joined by some of the tagged whales starting around 23 July.

All seven belugas whose transmitters lasted for 3 wk or more in 1992 or 1993 moved to the eastern coast of Devon Island and three moved north into Jones Sound. The most easterly recorded location was at 72°10′W.

Discussion

The timing of breakup of sea ice in the coastal areas of Somerset Island is variable and dependent on many different factors (Prinsenberg and Bennett 1987). The arrival of bel-

TABLE 6. Time spent in major estuaries, their adjacent waters, and in major geographical areas around Somerset

Tag No.	Total time, July–Aug.	Time in Cunningham Inlet	Time in grid offshore Cunningham	Time offshore Barrow Strait	Time in estuary north of Wadworth Island	Time in grid offshore estuary north of Wadworth Island	Time in Coningham Bay
1990							
5801		12.78	54.30	196.48	0		
5802		12.61	155.04		30.36	0	
5806		1.50	14.73	46.25	2.21	20.49	
8750	761.57	29.68	62.46	168.59	0	0	
8751	521.27	25.28	62.71	186.18	0	27.78	
1991							
5801	661.36	19.68	54.30	22.26			12.47
5805	575.00	15.80	16.02	6.31			14.86
8750	716	8.32	4.67	25.27	44.20	9.82	5.00
8757	33.23	1.17	4.62	9.72			
1992							
8752	853	7.65	8.02	26.61	15.68	64.28	0
8753	936	0.83	4.55	30.12	30.50	89.63	0
8754	920	0	6.41	74.70	77.25	23.70	0
8755	859	0	0.70	46.60	37.48	107.35	1.67

ugas at their traditional summer areas of aggregation is largely dependent on ice conditions (Smith et al. 1992), but was predictable within a range of about 14 d over a documented span of 12 yr.

The coast of Somerset Island and southern Prince of Wales Island in Peel Sound are the main areas of beluga aggregation during July and August, with some minor and transient use of the southern coast of Devon Island. Animals in Prince Regent Inlet appear to follow belugas from Cunningham Inlet into Peel Sound, as the ice melts and breaks up there in early to mid-August. The large numbers of belugas occupying the Creswell Bay area persist there into the first weeks of August. One of the three tagged belugas in 1993 moved into Peel Sound; the other two occupied Barrow Strait and moved directly to Lancaster Sound. The late tagging of belugas in Creswell in 1993 perhaps has not given us as clear a picture of their summer distribution as we might have obtained if animals had been captured in July.

There has been no indication that belugas from Creswell Bay ever use Bellot Strait to join the concentrations seen in southern Peel Sound. Finley and Johnson (1977) maintained a watch in this area and also failed to detect belugas using the Strait. This is surprising, since we have often seen narwhals and harp seals moving through this narrow channel.

There are six principal sites of aggregation for belugas, all of them the mouths of river estuaries. On the east coast of Somerset Island are Elwin Bay, Batty Bay, and, further south, the Creswell River estuaries. On the north coast is Cunningham Inlet in Barrow Strait. To the west in Peel Sound – Franklin Strait, is the small estuary north of Wadworth Island and the Coningham Bay estuary. Additionally, a few smaller streams and rivers are important, especially in the early part of summer while access to the main areas is blocked by ice. The small stream at Fellfoot Point near Maxwell Bay on Devon Island (74°30′N, 88°35′W) and several small rivers on the Brodeur Peninsula, Baffin Island, are frequented by belugas. During the summer, belugas briefly frequent the small river at Cape Anne (74°05′N, 94°44′W) on the north coast of Somerset Island, often after previously visiting Cunningham Inlet, a short distance to the east. The use of estuaries is related to a synchronous moult which is promoted by contact with warm freshwater (St. Aubin and Geraci 1989; St. Aubin et al. 1990; Smith et al. 1992).

Animals from Cunningham Inlet often proceed into Peel Sound by way of the estuaries north of Wadworth Island and Coningham Bay. Belugas tagged at Elwin Bay followed the same pattern without spending any substantial time at Cunningham Inlet. The actual use of the specified estuaries during July and August accounts for only about 8% of the total time, but belugas spend from 29 to 35% of their time in the areas adjacent to estuaries.

It appears that belugas move in and out of estuaries to exploit feeding opportunities offshore. Persistent deep diving by belugas in areas of Barrow Strait outside Cunningham Inlet in the Franklin Trench outside of Coningham Bay probably indicates the exploitation of deepwater prey (Martin and Smith 1992; Martin et al. 1993).

The movement into Peel Sound by most of the belugas tagged during this study might be directly related to feeding. The persistence of a floe edge could provide feeding opportunities on ice-associated fauna (Dunbar 1981). The presence of large numbers of harp seals and narwhals along these ice edges also leads us to believe that feeding is occurring and that it is an area of high food availability.

We do not know what triggers the apparent quick and directed movement of belugas northwards out of Peel Sound and eastward through Barrow Strait (Finley and Johnson 1977) and Lancaster Sound (LGL 1983). This appears to occur regularly in late August – early September. Belugas make transient use of certain bays as feeding areas along the south Devon coast. In late August – early September, large schools of Arctic cod have been sighted in the bays along the southwestern coast (Welch et al. 1992), where belugas and seabirds feed heavily on them. During their rapid migrations to the east, belugas regularly frequent Croker Bay (74°30′N, 83°20′W) and eventually stop in the area around Philpots Island. In October, some whales move

Island and in Parry Channel.

Time in grid offshore Coningham Bay	Time offshore Peel Sound	Time in Elwin Bay	Time in grid offshore Elwin Bay	Time offshore Prince Regent Inlet	Time in Batty Bay	Time in grid offshore Batty Bay	Time offshore Lancaster Sound	Timing between distant quadrats
				0				0
								18.45
	160.84							436.99
	58.73			2.52			0	439.92
	130.14							89.28
264.69	288.12							
176.89	340.92							420.13
181.17	351.25						59.0	
	41.67							
160.94	549.59	1.56	4.57	98.58	1.92	19.66	54.74	303.28
0	298.27	48.91	65.88	52.76	17.01	35.80	802.37	253.91
0	308.70	30.10	81.98	57.72	6.47	43.51	213.88	387.73
82.81	451.59	29.23	68.11	92.26	0	0	429.30	165.44

TABLE 7. Proportion of time spent in major geographical areas or moving between locations by UHF-tagged belugas between 24 July and 4 October 1991 and 1992.

	Tag No.	Total time tagged (h)	Estuaries or adjacent waters	Offshore Barrow Strait	Offshore Peel Sound	Offshore Prince Regent Inlet	Offshore Lancaster Sound	Moving
31 July – 13 Sept.	8750	1044	0.245	0.024	0.336	0	0	0.402
27 July – 17 Sept.	8752	1381	0.215	0.01	0.265	0	0	0.318
24 July – 4 Oct.	8753	1741	0.463	0.015	0.315	0.056	0.031	0.174
24 July – 26 Sept.	8754	1549	0.303	0.048	0.199	0.037	0.138	0.250
27 July – 28 Sept.	8755	1539	0.2127	0.030	0.293	0.059	0.278	0.107

TABLE 8. Dates of major movements of UHF-tagged belugas.

Date tagged	Tag No.	Arrived Peel Sound	Arrived Coningham Bay	Move to Barrow Strait	Move to Lancaster Sound
Cunningham Inlet, 1990					
15–20 July	5801				
16 July	5803	24 July			
23 July	5806	24 July			
15 July	8750	27 July			
15 July	8751	27 July			
Cunningham Inlet, 1991					
21 July	5801	25 July	29 July		
29 July	5805	31 July	3 Aug.		
31 July	8750	2 Aug.	5 Aug.	Lost in Peel Sound	7 Sept.
1 Aug.	8757	2 Aug.		30 Aug.	
Elwin Bay, 1992					
26 July	8752	5 Aug.	18 Aug.	5 Sept.	15 Sept.
28 July	8753	4 Aug.	17 Aug.	26 Aug.	30 Aug.
23 July	8754	5 Aug.	(Only to 72°53′N)	Lost in Peel Sound Sept.	
26 July	8755	7 Aug.	18 Aug.	5 Sept.	7 Sept.
Creswell Bay, 1993					
13 Aug.	8750	22 Aug.	28 Aug.	7 Sept.	12 Sept.
15 Aug.	8753			22 Aug.	24 Aug.
15 Aug.	8755			27 Aug.	31 Aug.

TABLE 9. Summary of travel speeds (km/h) from UHF-tagged belugas calculated from Argos-derived locations of quality ≥2 and time intervals between locations of ≥5 h.

	Tag No.	Avg. speed	No. of measuremens	No. of hours	SD	Max. speed
1990	5801	1.51	11	133.0	0.73	2.44
	5803	0.72	8	178.4	1.44	4.21
	5806	1.55	8	237.8	1.50	4.50
	8750	2.25	13	175.6	0.86	3.77
	8751	1.70	21	267.8	1.16	3.80
1991	5801	1.29	30	537.6	1.03	4.11
	5805	1.36	16	404.6	0.65	2.74
	8750	1.85	36	901.0	1.19	5.75
	8757	4.10	5	30.3	1.31	6.18
1992	8752	1.54	29	726.6	1.13	4.21
	8753	1.20	64	1451.8	1.36	5.51
	8754	1.17	46	1289.1	0.97	3.73
	8755	1.68	47	1192.1	1.20	5.20
1993	8750	1.76	9	588.5	1.19	3.58
	8753	1.17	4	140.2	0.73	1.75
	8755	0.75	4	757.3	0.51	1.33

north into Jones Sound and eventually some move west into Greenlandic territory where our last transmissions have been received. More information is required about the behaviour of belugas in this late autumn period, since no aerial surveys were conducted at that time.

One of the striking results of our study is the demonstration that belugas in the Canadian High Arctic maintain a well-defined pattern of movement from year to year. Furthermore, the consistency of the behaviour of the animals tracked by radio telemetry indicates that the pattern is adhered to by the great majority of the individuals in the stock. This picture became clearer and was reinforced whenever aircraft were used to search the area in which one or more of the radio-tagged animals had been located via satellite. In every case, hundreds or thousands of belugas were found in the vicinity. We are confident that even this small sample of tagged animals (in relation to the population size) has been adequate to describe in broad terms the behaviour of the population as a whole.

There is an urgent need to reassess the size of this beluga stock which is, in all probability, shared by Canada and Greenland. Recent winter surveys (Heide-Jorgensen 1994) indicate significant declines since our latest estimates of stock size in 1981 (Smith et al. 1985) because of the high continuing harvests. Belugas are difficult to count because of their clumped distribution, their ability to move long distances in a short time, and because they can spend a significant proportion of their time under water. The consistent patterns of movement and the inventory of all areas of seasonal aggregation documented in our study provide the detailed background needed to plan future surveys for population size estimates.

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PERSPECTIVES

Obstinate Nature: An Ecology of Individuals. Thoughts on Reproductive Behavior and Biodiversity

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Marine turtles, salmon, and many other marine and terrestrial animals exhibit similar reproductive strategies in which the adults return to the site of their birth ("natal homing"). For these species, imprinting is the basic mechanism that allows every individual to place its progeny in an environment geographically similar to the one experienced at an early life stage. A tentative generalization of "natal homing" is presented within which homing is viewed as part of a continuum of reproductive strategies, all relying on imprinting. This generalization postulates that a newborn individual memorizes early environmental cues, which later determine the choice of its reproductive environment. Thus, the same mechanism accounts for successive generations reproducing at the same geographic location (philopatry) or aiming at a moving target, i.e., a set of environmental conditions that do not always have the same earth coordinates (dispersal). As a consequence, the adaptability of a population to its environment is ensured because of the diversity and multitude of imprinted individuals and not because of their individual adaptability. Diversity of life is thus perhaps more impressive than usually thought. Some ecological and evolutionary consequences of this generalization are explored and its application to marine fish discussed.

Chez la tortue de mer, le saumon et bon nombre d'autres espèces animales marines et terrestres, on observe des stratégies similaires en matière de reproduction, où les adultes reviennent à l'endroit de leur naissance (« retour au bercail »). Pour ces espèces, l'empreinte est le mécanisme fondamental qui permet à chaque individu de placer sa progéniture dans un environnement géographiquement similaire à celui qu'il a connu au début de sa vie. Nous présentons une tentative de généralisation du phénomène de « retour au bercail », en le considérant comme une partie d'un continuum des stratégies en matière de reproduction, toutes basées sur l'empreinte. Le postulat de cette généralisation est que tout nouveau-né mémorise les premiers signaux environnementaux qu'il perçoit, lesquels détermineront plus tard le choix de son environnement reproducteur. Ainsi, le même mécanisme permet à des générations successives de se reproduire dans la même zone géographique (philopatrie) ou de viser une cible mobile, c.-à-d. un ensemble de conditions environnementales qui n'ont pas toujours les mêmes coordonnées terrestres (dispersion). En conséquence, l'adaptabilité d'une population à son environnement est assurée par la diversité et la multitude des individus imprégnés, et non par leur adaptabilité individuelle. Ainsi, la diversité biologique pourrait être plus impressionnante que ce que l'on pense en général. Nous explorons certaines conséquences écologiques et évolutionnaires de cette généralisation, ainsi que son application aux espèces de poissons marins.

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A Salmon Is a Salmon but Two Salmon are Different, A Turtle Is a Turtle but Two Turtles are Different...

Biological diversity in nature appears to be immense and is discouraging for anyone who might be tempted to describe it (May 1992). Thirty million or more species inhabit the earth; for those with sexual reproduction, every individual has a genetic uniqueness, i.e., has a unique behavior and a specific relationship with its environment. Within the same species, however, individuals display strong morphological, biological, and behavioral similarities. A salmon is a salmon and a turtle is a turtle, but what makes two salmon or two turtles different and nevertheless similar? To Mayr (1982), the differences between biological individuals are real, while the mean values which we may calculate in the comparison of groups of individuals are man-made inferences. The way we consider groups of biological entities and utilize these inferences has been, and still is, a controversial subject in ecology, particularly when referring to relevant scales of observation (Levin 1992). Ecologists

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presently know more about the elements of a system than about the dynamics of the system itself (Allen 1988; Lomnicki 1988). Even when it is possible to integrate some of the genetic, behavioral, or ecological differences into more realistic computer simulations (Huston et al. 1988), results often lead to enormous complexity in the model predictions (DeAngelis et al. 1986). More realistic models also generate patterns of extreme richness that have a mathematical and sometimes an aesthetic interest of their own (Nowak and May 1992), but most of the time, the potential implications for population dynamics remain unknown. As noted by Lomnicki (1988), "intrapopulation variability is the basis of the most general biological theory — the theory of natural selection — but this variability complicates enormously any mathematical description of ecological processes." However, processes operating at the level of individual organisms can determine properties at an upper level and consequently may play an important role in advancing our understanding in ecology (Koehl 1989; Judson 1994) and in evolution (Ghiselin 1992; Hull 1992).

Each species has its own innate and learned preferences when choosing a reproductive habitat: marine turtles lay their eggs on beaches, salmon spawn on gravel beds.... Within a species, individuals may respond to an incredible variety of specific environments (i.e., temperature, flow, substrate, depth, etc.), leading to a high diversity of reproductive habitats. However, in certain circumstances, patterns and processes can be identified which link individuals and their reproductive environments. This is the case when the mature female returns to the site of her birth to place her progeny in an environment similar to the one experienced at an early life stage. Philopatry appears to be quite common among aquatic and terrestrial species (pinnipeds, fishes, insects, etc.) (Baker 1978; McCleave et al. 1982; Papi 1992). However, many animals do not return to the site of their birth to find a place for their progeny (Greenwood 1980; Stenseth and Lidicker 1992). Who the dispersers are and why they disperse are two important issues of profound scientific interest (e.g., Johnson and Gaines 1990; Stenseth and Lidicker 1992). An equally important question is why do animals reproduce where they do? (Sinclair 1988). I present here the results of an effort to integrate different perspectives from ethology, ecology, and evolution to gain a more global understanding of the spatial dynamics of sexual reproduction. First, a review and a comparative analysis of the reproductive strategies among some marine animals that exhibit strong "natal homing" migration are offered. Then, a tentative generalization of "natal homing" is presented within which homing can be viewed as part of a continuum of reproductive strategies, all based upon some form of imprinting. Finally, some ecological and evolutionary consequences of this generalization are explored and its application to marine fish considered.

Some Strategies that Work: Wildlife Reproductive Migrations

Marine Turtles

Marine turtles, as a group, have changed little for millions of years (Lohmann 1992).What can be learned from these species that have survived so long through great environmental changes? Recently, they have been the subject of intense study because some of them are classified as endangered or threatened (Taubes 1992). Thus, their complex life history, particularly the migrations that were difficult to understand because of their spatial and temporal lengths, are now well documented. A brief review of the reproductive life history strategies of marine turtles may provide some answers to their evolutionary success.

Marine turtle species are found throughout the world's tropical and subtropical waters and are well known for their long-range breeding migrations (Carr 1986; Lohmann 1992). Turtles lay their eggs on beaches. As the hatchlings emerge from their nest, they confront a dangerous world: pursued by crabs, foxes, birds, and raccoons, the turtles scramble across the beach heading straight to the ocean, whether or not they are able to see it from their nest site, and plunge into the surf. Then, they swim until they find floating objects (e.g., a sargassum mat), crawl onto them, and go wherever the currents take them (Carr 1986). During this period, turtles cross hundreds to thousands of miles of apparently featureless ocean (Carr 1986; Lohmann 1992). Observed ages at first sexual maturity range from 9 to 58 yr (Bowen et al. 1992). In the case of *Chelonia*, as much as 50 yr may be necessary to reach breeding age. Then, somehow, they return to their breeding grounds (Carr 1986; Lohmann 1992). Much of what is known about the migrations between feeding and breeding grounds of marine turtles has come from tagging experiments on nesting females (Bowen et al. 1992). Mature females return to the same rookery for nesting in successive seasons (Carr and Ogren 1960). This led Carr (1967) to propose the "natal homing hypothesis" where the adult females return to nest at their natal rookery. For example, green turtles nest in colonies, and utilize specific beaches while adjacent habitats remain unvisited (Carr and Carr 1972). Of thousands of return visits that have been recorded among 28 000 tagged females, none has ever been seen on another potential nesting beach anywhere in the world (Carr 1986). Green turtles (*Chelonia mydas*) always return to where they nested before. Carr (1986) related an anecdote about a tagged female that, over 17 yr, returned 26 times to the same beach from feeding grounds at least 300 miles away and very likely farther. The average distance between her nest sites was a few hundred metres, and some of the nests were almost on top of one another. A consequence of such fidelity to site is that rookeries that have been extirpated over the last four centuries, mostly by native people or by navigators, have not been recolonized by natural recruitment (Parsons 1962).

"Natal homing", i.e., return to the beach of birth, is not the only mechanism that could permit this migration. Hendrickson (1958) and Owens et al. (1982) proposed an alternative "social facilitation" hypothesis, where first-time breeders follow experienced females to a nesting beach, and having had a "favorable" experience, fix on that site for future nesting. Bowen et al. (1992) noted that "philopatry to natal site does, however, generate the testable prediction that rookeries should exhibit genetic differentiation with respect to female-transmitted traits (such as mtDNA), whereas social facilitation would allow high rates of female-mediated gene flow between rookeries that share feeding grounds." Studies of genetic structures of the populations were recently undertaken (Meylan et al. 1990; Bowen et al. 1992). Differences shown in observed geographic structuring of mtDNA genotypes were consistent with the natal

homing hypothesis and indicated that social facilitation to a nonnatal site is either rare or nonexistent (Meylan et al. 1990; Bowen et al. 1992).

Thus, a strong natal homing for marine turtles is demonstrated, even if the mechanism is still not clearly understood. Genetically ordained homing to particular locations would presumably require generations of intense selection whereas imprinting would allow novel migratory circuits to be established in a single generation (Bowen et al. 1992). Recently, in light of the mtDNA separations of the rookeries within ocean basins, Bowen et al. (1989) suggested that imprinting on environmental cues, rather than site-specific genetic programming, is responsible for nest site choice. According to sea level changes associated with the Wisconsin glacial interval (Bowen 1978) or global cooling associated with Pleistocene glacial advances (Bowen et al. 1992), climate fluctuations alter the availability of turtle habitat quite rapidly on an evolutionary scale. Bowen et al. (1992) estimated that, for example, sites of present-day rookeries in Florida and Cyprus were probably too cool to support nesting even 10 000 yr ago. Considering the ephemeral nature of rookeries, these authors concluded that the new nesting beaches are certainly colonised by only one or a few gravid females, leading to rapid fixation of a mtDNA genotype in the neophyte rookery. In that case, colonization is an individual process and its establishment, in a single generation, is possible because of the spatial memorization gained through the imprinting process.

Salmon

Life histories of the Pacific and Atlantic salmon have intrigued man for centuries. Salmon possess unusually complex life histories by living both in fresh and salt water, precisely returning to their native river after extensive feeding migrations at sea, and realizing major morphological and physiological changes associated with sexual maturation. Widely studied because of their economic and recreational importance, the migratory behavior of seven species of the Pacific salmon (*Oncorhynchus* spp.) and of the only Atlantic species (*Salmo salar*) is now well documented (Groot and Margolis 1991; Pearcy 1992; Schearer 1992). After several months of larval incubation in stream or lake gravel beds, salmonid fry emerge and migrate within the stream. Salmon juveniles typically spend from a few months to many growing seasons in the stream before smoltification and enter the marine environment where they reside for one to several years (Groot and Margolis 1991). The timing and pattern of seaward dispersal are influenced by many environmental factors and, most probably, by the genetic characteristics of individual stocks (Heard 1991). Salmon appear to be almost continuous travelers and their movements at sea are complex. Their seasonal movements and feeding migrations take them to a number of different oceanic environments (Pearcy 1992). These migrations have never been consistently linked with major oceanographic features (Burgner 1991).

Pacific and Atlantic salmon return to their natal streams to spawn, one to several years after leaving the rivers as smolts (e.g., Stabell 1984; Groot and Margolis 1991). In contrast with the migrations at sea, the homeward migration of maturing fish is strongly oriented, rapid, and precise, since generally little straying has been registered in natural systems (Carlin 1969). Tag recovery patterns reveal that maturing salmon in the same ocean area at the start of their return migration may travel to widely different final destinations or that maturing fish, even though spread out over a broad geographical area and experiencing different environmental conditions, may migrate to the same coastal location (Burgner 1991; Heard 1991). Although timing of spawning varies little from year to year within a specific spawning area, there are great differences in timing among spawning areas, even within river systems where individual populations may spawn at widely different times (Burgner 1991; Healey 1991). The remarkable temporal precision of convergence on the mouth of their home stream and the high rate of travel, approaching maximum sustainable speeds, suggest strong orientation (Burgner 1991).

The salmon's "natal homing" ability is one of the best-studied migratory patterns among animals. Homing behavior has an innate and a learned component (McIsaac and Quinn 1988). Transplanting individuals from one area to another is an experimental way to test the ability of salmon to imprint different environmental cues. When eggs are transplanted from one stream to another, the adults, particularly wild fish, show a strong fidelity to the recipient stream. The "imprinting" hypothesis postulates that streams "contain some characteristic odors, to which the young salmon become conditioned (or imprinted) while in the stream, and which they recognize and orientate to, upon reaching the parent stream as mature migrants" (Stabell 1984). The sensitive period for the young salmon appears to be very short. An "imprinting" period of 36–48 h has been sufficient to assure a high degree of homing success for coho salmon (*O. kisutch*) (Sandercock 1991) and might occur as early as hatching in the case of pink salmon (*O. gorbuscha*) (Heard 1991). Morpholine imprinting experiments (reviewed by Hasler and Scholz 1983) also represent examples of the significant role played by early experience in the development of goal recognition. The alternative "pheromone hypothesis", originally developed by White (1934), suggested that the presence of young salmon in a stream is one factor that causes adult salmon to select their spawning stream. This mechanism appears to be analogous to the "social facilitation" hypothesis previously discussed for marine turtles. Thus, natal area recognition would be possible by an animal that is imprinted with unique stream odors (Hasler 1966) or recognition of stock-specific pheromones (Nordeng 1971; Stabell 1984). However, imprinting is the commonly accepted mechanism for explaining the very precise homing ability observed for salmonids (Brannon et al. 1984; Saglio 1986; Quinn et al. 1987).

Tag recovery data reveal that straying in natural salmon populations rarely exceeds 10% (Ricker 1962) and usually remains below 5% (Hasler and Scholtz 1983). As noted by Heard (1991), only when a fish has spawned can a judgment be made as to whether it has homed or strayed; otherwise there is still the possibility for the fish to migrate elsewhere and to retrace its route and return to its natal river. Some recent and more unequivocal studies estimated that straying rates may be less than 1.5% for spring chinook salmon (*O. tshawytscha)* (Quinn and Fresh 1984) or even less than 1% for sockeye salmon (*O. nerka*) (Quinn et al. 1987). Recoveries of coded wire tagged salmon suggest that most straying rates for wild coho salmon stocks were in the range 0.1–2% (Sandercock 1991; Labelle 1992).

Straying rates are low and do not appear to be greatly influenced by "adverse" or "favorable" environmental conditions. Three examples illustrate the strength of "natal hom-

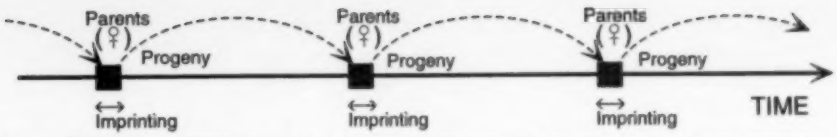

FIG. 1. From one generation to the next, individuals avoid the experience of new reproductive environments: they try to replicate the environmental conditions that they have gained through the teleonomic and irreversible imprinting mechanism (or similar early memorization of environmental cues).

ing" and the resulting lack of adaptability at an individual level. The first one is a natural environmental event that caused a spawning ground to become inappropriate for salmon. During the great Alaska earthquake on March 27, 1964, the intertidal portion of a streambed subsided 2 m in elevation (Roys 1971). An impassable falls, present before and after the earthquake, was located just upstream from the productive preearthquake intertidal spawning gravels. During the first postearthquake spawning period, a significant number of pink salmon spawned at the new lower 0.6–1.2 m tide level (preearthquake 2.4–3.0 m tide level) where eggs cannot survive because of continuous high-saline exposures. The second example is where anthropogenic changes of the environment physically obstruct the salmon's run. White (1936) transplanted Atlantic salmon fry into the east branch of Apple River, Nova Scotia, in 1932. Two tributaries of this river originally contained salmon. Some 60 yr before this experiment, a dam was erected forming a barrier to any spawning run of salmon. This dam had been out of use for 10 yr previous to the transplant, but salmon did not become reestablished in the stream until the successful transplant. Temporary obstacles do not prevent salmon from making repeated attempts to enter the native tributary, where they die instead of choosing an alternative spawning area (Ricker and Robertson 1935; Hartman and Raleigh 1964). The third example is derived from the dynamics of salmon itself and from density-dependent effects on its spawning grounds. Thus, Dvinin (1952), like many other authors, related that an exceptionally large and crowded accumulation of fish on the spawning grounds causes prespawning mass mortalities. From these examples, it is clear that adverse or favorable environmental changes do not affect what appears to be a quite "rigid" natal homing migration pattern.

Salmon exhibit high genetic variation within the same stream. Polygenetic variation found in some salmon protein systems, together with allele frequencies, indicates that the populations of different river systems are genetically distinct (e.g., Stabell 1984). A stream system perpetuates multiple subpopulations that can also be distinguished from each other by morphological characteristics, stage and age of maturity, time of entry into the river system, and localization of spawning regions. Nickerson (1979) suggested that genetic differences in early and late pink salmon spawners were such that "even minor straying would tend to homogenize gene frequencies in a few generations" and interpreted this as evidence of "little or no straying" between populations. Studying wild Atlantic salmon, Stahl (1982) estimated that to maintain this genetic homogeneity within subpopulations of 2500–10 000 individuals, the effective migration rate between the populations has to be less than one individual per year. The phenomenon of homing in anadromous salmonids, coupled with natural selection in response to environmental variation, has resulted in the development of numerous stocks, thereby maintaining a more or less discrete gene pool.

The First and Last Chance: The Lessons of Survival and Instinct

Learning from What is Possible

Marine turtles and salmon have their own life history traits and each group experiences a complex series of major physiological and habitat changes. However, marine turtles, salmon, and many other marine and terrestrial animals exhibit similar reproductive strategies in which the adults return to the site of their birth ("natal homing") (Baker 1978; Papi 1992). Their reproductive migrations are strongly oriented, rapid, and precise, bringing them to a specific location. During its migration upstream, a salmon spends an incredible amount of energy to reach the spawning grounds of its native stream; a marine turtle travels through the ocean to come back to the same native beach. Using sophisticated orientation abilities, these animals return very accurately to their natal sites. Tens to thousands of miles are traveled by animals to find a place for their progeny.

Individuals respond poorly, in terms of adaptability, to changes in their spawning habitat. If for any natural or anthropogenic reason the spawning site becomes unsuitable, the individuals do not attempt to breed at alternative areas, thereby condemning most of their progeny. Imprinting on the natal site appears to be the driving mechanism of these migrations and alternative hypotheses are considered ancillary (i.e., the "social facilitation" or the "pheromone" hypothesis). New reproductive environments are rarely experienced, except by a very low percentage of a population. However, imprinting permits the fixation, in a single generation, of new possible reproductive locations found by strays. How could such an apparent rigid reproductive strategy have operated for thousands to millions of years? In the following paragraphs, these facts and their importance in population dynamics are discussed from different ethological, ecological, and evolutionary perspectives.

Obstinate Nature

Reproduction is an essential event in an animal's life. Thus, a salmon that dies after spawning has only one chance to pass its gene to its offspring (multiple spawners have a few more chances!). The environment is usually adverse for individuals, particularly during the early life stages where mortalities are always at their highest. Surviving is difficult and the possibilities for an organism to complete its life cycle are definitively rare. In the marine environment, spatial structure is no doubt of critical importance in population regulation (Sinclair 1988). A mature female must carefully select an appropriate spawning site in an environment that is generally hostile and capricious. Marine turtles or salmon try to maintain what has "worked" for them at an individual level. From one generation to the next, individuals are risk adverse and avoid the experience of new

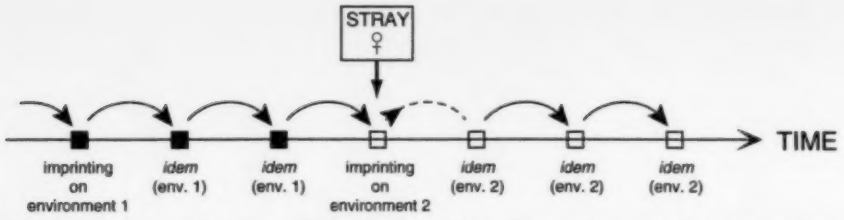

FIG. 2. Parents are producing "environmentally conditioned" descendants and the imprinting from one generation to the next is similar (imprinting on environment No. 1). When the female strays, the egg is then producing a new "environmentally conditioned" adult (imprinting on environment No. 2) (note the position of the arrows). Inertia and innovation both coexist in the imprinting process.

reproductive environments. Fidelity to the native site is strong and individuals are obstinate in not considering alternative reproductive sites. This way, many other possibilities are not explored and perhaps will always be ignored due to the apparently poor degree of adaptability of the "environmentally conditioned individuals". An animal that is looking for proper environmental conditions for its descendants is not considering environmental possibilities that will maximize its reproductive output. Their individual behavior is much more conservative. A return to the parental spawning ground provides a mechanism for maintaining survival by the repeated usage of a possible site. This environmental choice that is imprinted from one generation to the next has been experienced during many, many generations (Fig. 1): this is a possible and tested choice. Nature is obstinate and this obstinacy has evolutionary consequences.

An Evolutionary Interpretation of Imprinting

Darwin (1859) considered instincts as complex reflexes that have been produced through natural selection. Imprinting ("Prägung") is a passive learning process that is closely linked to the development of instincts (Lorenz 1970). During a genetically defined critical period, an individual has the ability to memorize an "insight" into its environment, i.e., particular cues or sign stimuli that are perceived in the surrounding environment of the offspring. Imprinting is characterized by its short duration in time and by its irreversibility: it is not possible to modify it through a learning process that would change its adaptive value (Lorenz 1970). It is a teleonomic process (Fig. 1) where "the animal perceives the 'imprinting' stimulus in relation to something (a background) and develops a preference to that stimulus through its relative stimulation" (Stabell 1984). The mechanism of imprinting among animals has been clearly established for many animal taxa like insects, birds, fish, and mammals (McFarland 1981) and has been related to various environmental conditions (e.g., physicochemical, social). Imprinting has both short- and long-term implications: it works at the beginning of an animal's life as a way to recognize members of close kin and later for the choice of mate (Bateson 1979; McFarland 1981). Imprinting and other early memorization of environmental cues are complex mechanisms that actually play an important role in sexual reproduction (Lorenz 1970; ten Cate 1989).

When it emerges from its natal site, a turtle or a salmon knows nothing about its immediate environment, and must learn very quickly. It must assure its immediate survival as an individual in a contemporaneous ecological situation but also in an evolutionary context with a variable or changing environment. As nature is adverse and only few opportunities allow survival, natural selection has favored the ability to memorize some early environmental and/or social conditions that define a viable environment. This information is required for a surviving individual that later tries to find a place for its progeny.

As many different learning and instinctual processes are involved at the beginning of life, imprinting represents a rigid way to memorize some essential environmental and/or social cues. However, placing progeny in the same site is an "all-in-one" mechanism (or "template learning") that solves many problems arising at the beginning of life. First, this maintains patterns and structures by enabling mature adults to return to similar and viable environmental conditions, thus also increasing the probability of encountering a mate. It also provides the necessary environment during the early ontogenic interval. At this time the young often does not look like the adult and usually has completely different biological requirements. Thus, imprinting gives some chance of probable success in an uncertain world.

Strays do not contribute to the next generation of their source population. Different names or terms are found in the literature to characterize strays: "vagrants" in marine literature (Sinclair 1988), "emigrants", "gravid waifs" in the ecological literature (North 1988; Bowen et al. 1992), "expatriates" in the plankton literature, "error makers" in modeling (Allen 1988), etc. These individuals are not numerically important within a population but should be considered as essential in an evolutionary perspective (Quinn 1985). As noted by Bowen et al. (1992), an "absolute natal homing would be the recipe for extinction." It is not yet clear whether the percentage of strays within a population is due to some individuals reacting differently to the environment or to a low individual rate of errors in the recognition process of the environmental pathways that lead to the return to a new reproductive site (Dodson 1988).

Imprinting plays an essential role in population dynamics but maintaining the relationships between the individuals and their reproductive environment, but conversely, it permits the fixation, in a single generation, of new possible reproductive locations (Fig. 2). Thus, strays are essential for long-term dynamics by exploring and fixing new environmental solutions that later may become possible for the species. Exploring new environments leads to waste, most of the time, because the offspring will not survive. But at an evolutionary scale, this is a necessary waste and, from time to time, new possible environmental sites will be discov-

ered by strays. The imprinting process also has a dualistic character (Fig. 2): parents are producing "environmentally conditioned" eggs in an environment similar to the one where they were imprinted whereas when they stray, the egg is then producing a new environmentally conditioned adult. This is also an endless process where inertia and innovation both coexist and define each other.

A Tentative Generalization of Natal Homing: the "Éternel Retour" Hypothesis

Philopatry is common among animals (Baker 1978; Papi 1992); however, many animals disperse for reproduction and do not return to the site of their birth to find a place for their progeny (Johnson and Gaines 1990; Stenseth and Lidicker 1992). In that case, how does an individual choose its reproductive environment? To what extent is it an innate or acquired choice, i.e., a choice dictated by adaptive strategies elaborated by the species or, as for a salmon or a marine turtle, an individual experience that has been memorized at an early life stage? Marine turtles or salmon may be viewed not only as singular cases, but as cases of a more common mechanism regulating the spatial dynamics of reproduction. The "éternel retour" (perpetual return) hypothesis[1] represents a generalization of "natal homing" within which homing is viewed as part of a continuum of reproductive strategies all relying on imprinting. The "éternel retour" hypothesis postulates that a newborn individual memorizes early environmental cues, which later determine the choice of its reproductive environment. The same mechanism may account for why successive generations reproduce at the same geographic location (philopatry) or aim at a moving target, i.e., a set of environmental conditions that do not always have the same earth coordinates (dispersal). For that latter case, a well-defined ecological pattern is then difficult to disentangle at a population level.

The individual has an ethological, an ecological, and an evolutionary dimension and is at the heart of the "éternel retour" hypothesis. Several other conditions are required to build a viable dynamic, e.g., to define how individuals are "spatio-temporally localized cohesive and continuous entities (historical entities)" (Hull 1992), and why as "reproduction is a spatio-temporally localized process — parents and offspring must have some spatio-temporal continuity" (Ereshefsky 1992). The "éternel retour" hypothesis thus comprises three components that are required when defining a viable dynamic:

(i) *A conservative mechanism.* Imprinting or early memorization of environmental cues allows the fixation within a generation of environmental reproductive possibilities. What has been possible during many generations at an evolutionary scale or what is newly experienced in the environment (by reference to the existing pattern) persists.

(ii) *Obstinacy at an individual level.* The strength of the imprinting or other early life memorizations allows the maintenance of the environmental possibilities for many generations and the ignorance of contemporaneous changes (as imprinting is not altered by any behavioral or environmental information).

(iii) *A small percentage of strays.* Few individuals within a population stray or a low rate of error at an individual level is necessary to experiment with new, possible solutions that are generated by environmental changes.

Some ecological and evolutionary consequences of this generalization are explored in the next paragraphs.

An Ecology of Individuals: Some Ecological and Evolutionary Implications

Every individual is different through the teleonomic and irreversible mechanism of imprinting. Within a population, two individuals are different from a reproductive point of view in that they will not make exactly the same choice of a reproductive site according to their imprinting. The imprinting mechanism appears to be able to maintain but also to diversify patterns as it concerns individuals and their relationships with the environment. As a direct implication, adaptability of a population to its environment is ensured because of the diversity and the multitude of imprinted individuals and not because of their individual adaptability (Jacob 1981). Whereas the adaptability of the imprinted individuals is poor, populations are nevertheless constituted of hundreds, thousands, or millions of different individuals, each of which acts differently, searching for a specific environment to reproduce according to innate habitat preferences and a previously acquired norm during ontogeny.

It is currently accepted that individuals of a given population try to select and track the optimal environmental conditions that maximize its total reproductive output; thus, it is generally thought that a species should have a "best" reproductive strategy (Potts and Wootton 1984). A demographic exchangeability of individuals (two individuals are then equivalent) is used to define populations in most models of population and community ecology (Templeton 1989). Conversely, the concept of an individual-based population dynamic permits us to consider an ecology of possibilities versus an ecology of optima. Populations live in an heterogeneous and changing environment where many reproductive sites are possible but not optimal for survival at an individual or at a population level (although this may be the case). A surviving individual that has experienced specific environmental conditions and that attempts to spawn under similar conditions may be able to improve the overall reproductive value of the population, but the converse may also occur because many other environmental possibilities are ignored. It is quite clear that the optimization approach to improving our understanding of reproduction presents many conceptual difficulties. If optimization is one of the most powerful and elegant tools for understanding adaptations (Parker and Maynard-Smith 1990), inherent problems come into view when considering an ecology of possibilities versus an ecology of optima. At an individual level, there may also be possible choices.

Under the assumption of demographic exchangeability, one tends to focus on mechanisms that create diversity or isolation among populations. Conversely, when one admits that all individuals are different, proximity or cohesion mechanisms between individuals become key concepts for defin-

[1]Eliade (1969) introduced the "éternel retour" as a myth in traditional societies. He conceives archaic people as perceiving reality through an archetype experienced by others (i.e., a myth). An individual's life appears to be an endless repetition of gestures that have been originated by others and every act has a meaning by reference to a preexisting model. Reality is thus defined as an ontological archaism that leads to reject contemporaneous events that were not previously defined.

ing the identity of groups. Under an individual-based ecology, a population can be defined as a group of individuals that have similar imprinting and that are actually able to breed in the same geographical location. Recognizing the importance of the individuals should lead to the consideration of factors that permit cohesion between them instead of isolating factors that diversify homogeneous population.

An individual-based ecology also leads to a different view of how environmental changes may affect population dynamics. As noted by Lewontin (1983), "by its behavior — its genetically determined use of habitats and resources — an animal largely defines the selective pressures to which it is subject." Environmental changes cause selection among imprinted individuals. Thus, selective pressures should be particularly strong at an individual level as they lack adaptability. Nature works more easily by rearranging what it has than by constructing novelty (Gould 1992) and qualitative changes within a population are certainly constantly acting even under minor quantitative changes. This may lead to a view similar to the Red Queen hypothesis (Van Valen 1973) where qualitative changes within a population are constantly acting on an ecological time scale.

Sympatric speciation may have a much more significant role in evolution than previously thought (Otte and Endler 1989; Ereshefsky 1992) and cohesion mechanisms (as opposed to isolation mechanisms) are now regarded as important factors regulating populations (Sinclair 1988; Templeton 1989). Specific-mate recognition systems (Paterson 1992) or the role of habitat preference (Diehl and Bush 1989) creates intrinsic barriers that act even when a population resides in the same place; this should lead to geographic speciation. Imprinting may work like an "invisible barrier" between individuals that share the same ecosystem and only have the potential to reproduce with a few other individuals. An imprinting process that conditions individuals and consequently diversifies populations would represent a process acting on an ecological time scale that may lead to sympatric speciation.

The "éternel retour" hypothesis will hopefully provide a conceptual framework for organizing our knowledge of ecological patterns. It should give some new insights and refresh our views in fish population dynamics.

Applications to Marine Fish Populations

Many species of fish are well known for their homing migrations, e.g., Atlantic herring (*Clupea harengus*), capelin (*Mallotus villosus*), American shad (*Alosa sapidissima*) (Harden Jones 1968; Baker 1981). Traditional knowledge of the Sami fishermen states that the fjord cod (*Gadus morhua*), like salmon, migrate back to their exact place of birth to reproduce. Local Sami taxonomy recognizes different spawning stocks of cod; the Sami also know that overfishing of local stocks may result in total abandonment of certain spawning sites (Eythorsson 1993). Scientific knowledge recognizes the importance of homing for cod (Lear and Green 1982; Jakobsen 1987) and the evidence of genetically discrete populations of haddock (*Melanogrammus aeglefinus*) in the North Atlantic (Jamieson and Birdley 1988; Zwanenburg et al. 1992). However, our understanding of the patterns and particularly the mechanisms of homing in fishes is derived from research on a very small number of species that reproduce at specific spawning grounds (Quinn and Dittman 1992). Small pelagic fish, particularly in upwelling areas, move into a disturbed habitat and do not have particularly regular reproductive migrations (Blaxter and Hunter 1982). Thus, in a changing environment with no persistent and spatially defined landmarks, one should not expect to observe natal homing migration patterns. In this section, I bring together some indirect and specific "knowns" into a broader framework promoting the idea that pelagic species may be using the same imprinting process as salmon to "home" to a moving target, i.e., a set of environmental conditions that are not always located precisely in earth coordinates.

Ecologists have been working for decades collecting environmental and biological data in pelagic ecosystems. An impressive literature is now devoted to analyzing the relationships between quantitative changes in pelagic fish populations and the environment (e.g., Sharp and Csirke 1983; Pauly and Tsukayama 1987; Cury and Roy 1989, 1991; Kawasaki et al. 1991). Even though major quantitative changes have occurred over the last decades in most marine systems, it is still very difficult to identify consistent patterns between populations and their environments (Laster and MacCall 1983; Cury 1988). In studying the relationship between fish abundance and environmental conditions, one often assumes that fish select the most suitable environmental conditions. If this were the case, there would be a straightforward relationship between fish dynamics and environmental conditions. However, ecosystem predictability is poor and new theoretical considerations are needed. Field and genetic studies greatly modify any simplified view of population dynamics and structures. Thus, despite the ability to induce spawning in some species, Blaxter and Hunter (1982) recognized that we do not understand what environmental factors determine the onset and cessation of spawning in the sea. Hedgecock et al. (1989) reported genetic heterogeneity within the central California northern anchovy (*Engraulis mordax*) stock, previously regarded as a panmictic population. Thus, anchovy stocks that were thought to be homogeneous are in fact a mosaic of "elementary" populations (sensu Lebedev) (Hedgecock 1991). According to Mathisen (1989) the anchoveta (*Engraulis ringens*) stock off Peru consists of a large number of local subpopulations each of which is genetically adapted to "home" to a different upwelling location and to spawn during a specific period of the year. European sardine populations are traditionally assigned to six different "races"; however, further subdivisions have been made according to spawning seasons that are separated by several months and that may define sympatric populations (Wyatt et al. 1991). Many examples can be found in the literature that emphasize the fact that fish populations are heteregeneous, even in an apparently homogeneous environment, and that this heterogeneity should play a fundamental role in population dynamics. As noted by Hedgecock (1991) for *Engraulis mordax* off California, the "demonstration of sympatric, biologically distinct subpopulations within the main spawning area of the central stock is unexplained by current concepts of northern anchovy stock structure."

Alternative views for marine population regulation recently have been developed that take into account qualitative changes and spatial constraints. In his essay on population regulation, Sinclair (1988) considered that life cycles and population patterns of sexually reproducing animals are

defined in relation to particular geographical or spatial constraints that ensure persistence. Sinclair determined two sets of constraints for sexually reproducing populations: one generated ultimately by energy and one by spatial structure, each of which defines a distinct selection process (i.e., "food-chain" and "life-cycle"). Spatial processes predominate in marine population regulation and many examples support the discrete population concept (Sinclair and Iles 1989). The "éternel retour" hypothesis recognizes the importance of spatial constraints for sexual reproduction but also emphasizes the role of learned components at the level of the individual. Each individual pelagic fish may tend to find in its contemporaneous reproductive environment the conditions it imprinted as egg (or larva?). This has in fact been speculated for Atlantic eels (Kleckner et al. 1983; McCleave et al. 1987) and for herring (Corten 1993). The reproductive strategies of marine turtles, salmon, herring, capelin, sardine, or tuna are thus perhaps more similar than suspected.

Future Research on Reproductive Behavior

More studies of population structure on fine spatial and temporal scales are needed to answer questions such as How much of learned and innate components are involved in the spatiotemporal reproductive dynamics of a species and how will a mosaic of elementary populations respond to environmental changes or to changes in exploitation rates? Defining the degree of adaptability at an individual level should receive more attention in ecological and evolutionary studies, as it has fundamental consequences on short- and long-term ecosystem predictability. Working or testing ideas at an individual level has received little attention in ecology (Lomnicki 1988; Judson 1994) and null hypotheses that can be tested are needed (Peters 1991). Hopefully, the "éternel retour" hypothesis will provide a heuristic framework for organizing our thinking and our knowledge about spatial aspects in sexual reproduction. Collaborative efforts between ethologists, ecologists, and evolutionists are needed to better understand the mechanisms that diversify and create heterogeneity in marine populations as well as to improve our ability to predict quantitative changes in fish populations.

Life has always evolved in a sea of unknown and yet has prospered (Holling 1978). As soon as it emerges, a newly born animal is fragile and must face the unknown including the adversities of an always changing environment. Consequently, when mature, the surviving individual must find a proper environment for its progeny. The survival of the species is engaged with an indifferent player: the environment. If this evolutionary game is about "what to do as the future is unknown", a possible answer given by the "éternel retour" hypothesis is "doing the same". As a response to risk adversity, inertia represents an important component in the functioning of natural systems (Aubin 1991). Because every individual is able to explore a specific environmental possibility and in some way to transmit it to its descendants, it makes each of these individuals unique in terms of their reproductive behavior. Diversity of life is perhaps even more impressive than usually thought.

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ANNOUNCEMENTS/AVIS

Fifth International Symposium on the Reproductive Physiology of Fish

Austin, Texas (USA)

A symposium on the reproductive physiology of fish will be held at the main campus of The University of Texas at Austin on July 2–8, 1995. This symposium will provide an excellent opportunity for scientists interested in all aspects of fish reproductive biology to interact and exchange information on the most recent advances in this field. The symposium will consist of oral sessions, poster sessions, and demonstrations. Session topics will include a variety of research topics such as gonadal physiology, gametogenesis, hypothalamic–pituitary axis, behavior, reproductive life history, environmental influences on reproduction, and aquaculture. Proceedings based on oral and poster presentations will be published as short papers in book form shortly after the meeting. Accommodations and full meal services will be available at affordable prices on campus, and a Texas-style social program for participants and guests is also planned. Limited financial assistance for junior scientists is anticipated. Deadlines: a call for abstracts will be issued in **fall 1994**, and abstracts will be due in **February 1995**. Contact: Dr. Peter Thomas, The University of Texas at Austin, Marine Science Institute, P.O.B. 1267, Port Aransas, TX 78373, USA.

Cinquième symposium international sur la physiologie de la reproduction des poissons

Austin, Texas (É.-U.)

Un symposium sur la physiologie de la reproduction des poissons aura lieu sur le campus principal de l'Université du Texas à Austin du 2 au 8 juillet 1995. Les chercheurs intéressés à tous les aspects de la biologie de la reproduction des poissons auront là une excellente occasion d'échanger de l'information sur les plus récents progrès réalisés dans ce domaine. Présentations orales, séances d'affiches et démonstrations sont au programme. On traitera d'une grande diversité de sujets de recherche comme la physiologie des gonades, la gamétogenèse, l'axe hypothalamique–pituitaire, le comportement, le cycle reproducteur, les effets de l'environnement sur la reproduction et finalement l'aquaculture. Les comptes rendus des présentations orales et des séances d'affiches seront publiés dans un volume sous forme d'articles courts peu après le symposium. Des services d'hébergement et de restauration sont offerts à prix abordables sur le campus même et un programme d'activités sociales à la mode texane est planifié à l'intention des participants et des invités. Une aide financière limitée est prévue pour les jeunes chercheurs. Dates de tombée : l'appel de résumés aura lieu à **l'automne 1994** et la date de réception des résumés est fixée à **février 1995**. Contact : D[r] Peter Thomas, The University of Texas at Austin, Marine Science Institute, P.O.B. 1267, Port Aransas, TX 78373, USA.

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Editor-in-Chief **H.J. Dumont**

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